7.75 (2H, m), 7.73-7.3 (8H, m), 7.25 (2H, m), 7.21-6.7(6H, m), 5.4-4.8(6H, m), 4.78-4.21 (4H, m), 3.98 (3H,s), 2.1-1.75 (8H, m), 1.55 (3H, m), 1.28(3H, m), 0.99(6H, m).

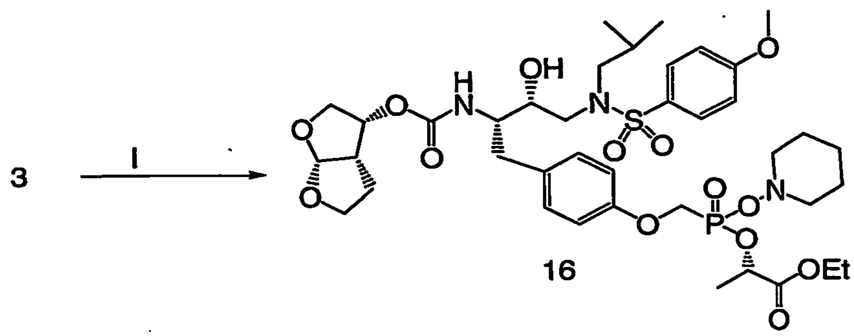
Example 14

Compound 14: To a solution of compound 5 (0.770 g, 0.790 mmol) in dichloromethane (10 mL), under ice-cooling, was added triflouroacetic acid (5 mL), the resulting mixture was stirred at 25°C for two hours. The reaction mixture was concentrated under reduced pressure and the residue was co-evaporated with EtOAc to provide an yellow oil. To a solution of the above oil in (10 mL) of EtOAc, under ice-cooling and stirring was added formaldehyde (210 µL, 2.86 mmol), acetic acid (252 µL, 4.30 mmol), followed by sodium cyanoborohydride (178 mg, 2.86 mmol). The mixture was further stirred at 25°C for 2 hours. The above mixture was concentrated and diluted with EtOAc and washed with H_2O (3X), brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using reversed-phase HPLC to provide 420 mg of compound 14: ^{1}H NMR ($CDCl_3$) δ 7.8(2H, d, J=8.9Hz), 7.4 (2H, m), 7.3-6.8 (8H, m), 5.75 (1H, m), 5.3-5.1(2H, m), 4.6-4.23 (4H,m), 3.98 (3H, s), 3.7-2.6 (15H, m), 2.2-1.8 (8H, m), 1.72 (3H, s), 1.58(3H, m), 1.25 (3H, m), 0.95 (6H, m).

Example 15

Compound 15: To a solution of compound 6 (100mg, 0.114 mmol) in EtOAc (1 mL) was added 1-Methyl-piperazine (63.2 mg, 0.570 mmol), acetic acid (34.0 µl, 0.570 mmol) followed by Sodium Cyanoborohydride (14.3 mg, 0.228mmol). The mixture was stirred at 25°C for 14 hours. The reaction mixture was concentrated and diluted with EtOAc and washed with H_2O (5X), brine (2x), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH_2Cl_2/Isopropanol= 100/6.5) to give 5.22 mg of compound 15: ^{1}H NMR ($CDCl_3$) δ 7.73 (2H, d, J=8.9 Hz), 7.4-7.18(8H, m), 7.1-6.89 (2H, m), 5.67 (1H, m), 5.2-4.9 (4H, m), 4.30-4.11 (4H, m), 3.98 (1H, m), 3.89 (3H, s), 3.87 (1H, m), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (10H, m), 2.80-2.25 (8H,m) 1.65(2H, m), 1.86 (1H, m), 1.6(3H, m), 1.30(3H,m), 0.92(6H, m).

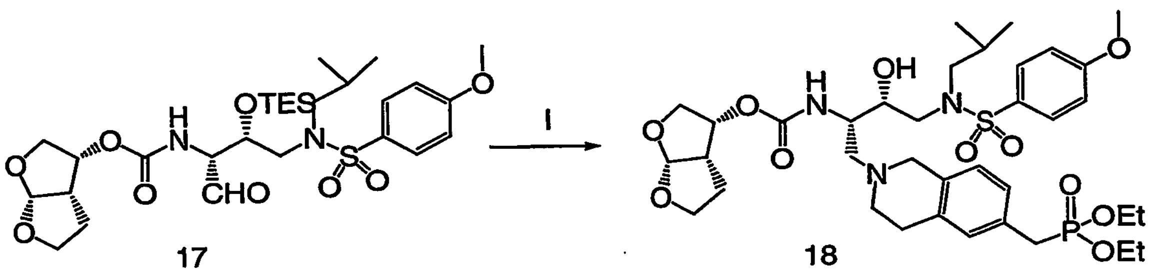
Scheme 5



16

I.Piperidin-1-ol/DCC/Pyridine

Scheme 6



17 18

I. a:R_2NH /HOAc/$NaBH_3CN$/EtOAc b: 2%HF/CH_3CN

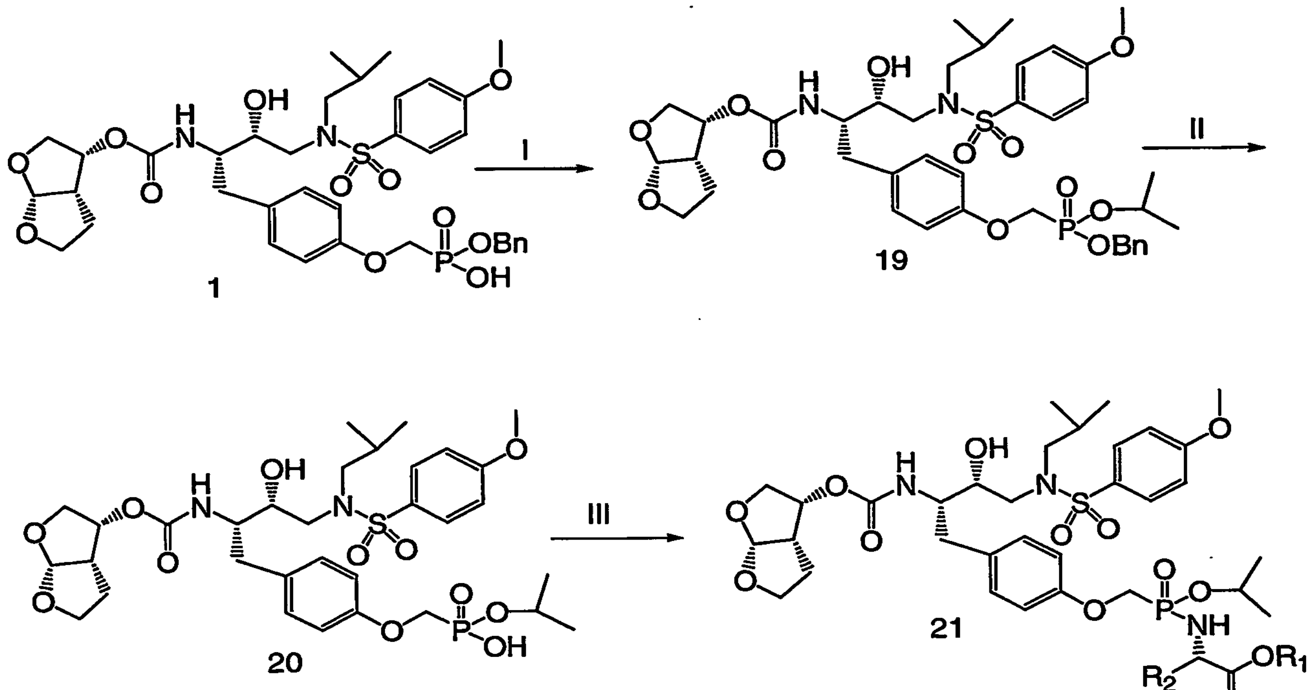
Example 16

Compound 16: To a solution of compound 3 (100mg, 0.120 mmol) in Pyridine (600 µL) was added Piperidin-1-ol (48.5 mg, 0.480 mmol), followed by N,N-Dicyclohexylcarbodiimide (99.0 mg, 0.480 mmol). The mixture was stirred for 6 hours, the solvent was concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/Methanol= 100/5) to provide 17 mg of compound 16: ^1H NMR ($CDCl_3$) δ 7.73 (2H, d, J=8.9 Hz), 7.16 (2H, m), 7.0 (2H, m), 6.9 (2H, m), 5.68 (1H, m), 5.17 (1H, m), 5.04 (1H, m), 4.5-4.2 (4H, m), 3.90 (3H, s), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (10H, m), 2.80(3H, m) 1.65(2H, m), 1.86 (1H, m), 1.6(3H, m), 1.5-1.27 (9H,m), 0.92(6H, m).

Example 17

Compound 18: To a solution of compound 17 (148 mg, 0.240 mmol) in 4 mL of Methanol was added (1,2,3,4-Tetrahydro-isoquinolin-6-ylmethyl)-phosphonic acid diethyl ester (70.0 mg, 0.240 mmol), acetic acid (43.0 μL, 0.720 mmol). The reaction mixture was stirred for 3 minutes, followed by addition of Sodium Cyanoborohydride (75.3 mg, 1.20 mmol). The reaction mixture was stirred at 25°C for 14 hours. The reaction mixture was diluted with EtOAc and washed with H_2O (3X), brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH_2Cl_2/Isopropanol= 100/5) to give 59 mg of TES protected intermediate. 83 μL of 48% HF solution was added to acetonitrile (4 mL) to prepare the 2% HF solution. The above 2% HF solution was added to TES protected intermediate (47 mg, 0.053 mmol) and the reaction mixture was stirred for 2 hours. The solvent was concentrated and the residue was diluted with EtOAc, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH_2Cl_2/Methanol= 100/10) to give 35.2 mg of compound 18: ^1H NMR ($CDCl_3$) δ 7.73 (2H, d, J=8.9 Hz), 7.05 (2H, m), 6.89 (2H, m), 6.76 (1H, m), 5.75 (1H, m), 5.67 (1H, m), 5.3 (2H, m), 4.2-3.6 (12 H, m), 3.4-2.4 (11 H, m), 2.1-1.8 (6H, m), 1.4-1.28 (8 H, m), 0.92(6H, m).

Scheme 7

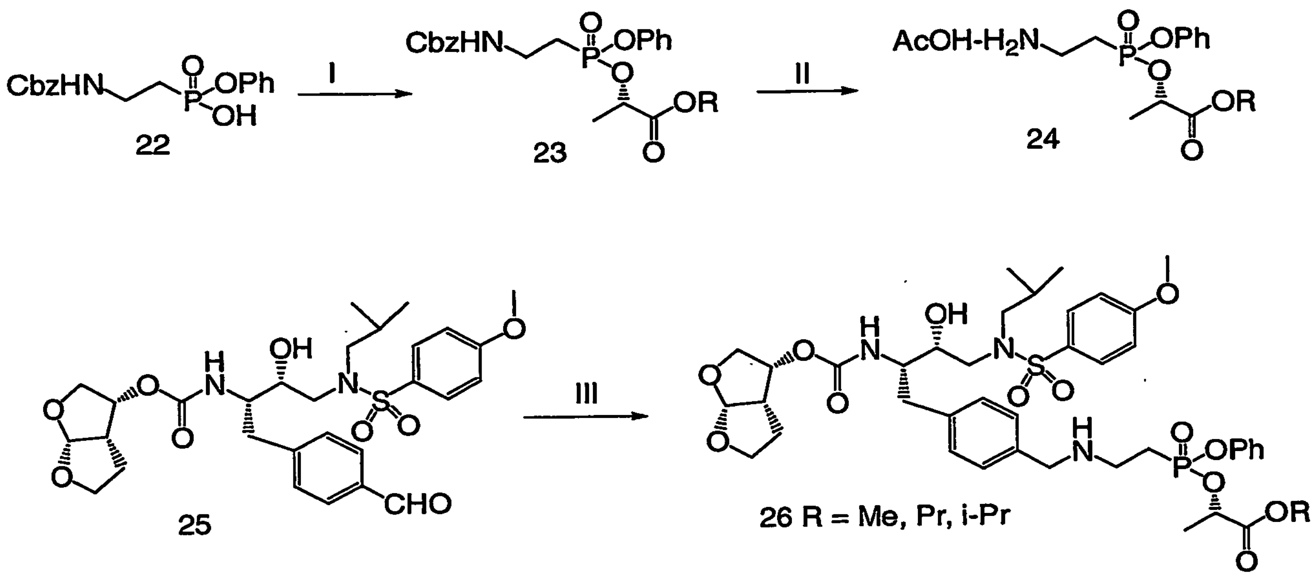


R_1 = Me, Et, i-Pr
R_2 = Me, Et, i-Pr

I. Isopropanol/Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate/ DIPEA/DMF;
II. H_2/10%Pd-C/EtOAc-EtOH;
III. RNH_2/Aldrithiol-2/PPh_3/iPr_2NEt/pyridine

Compound 19 is prepared following the procedure for compound 2 by using monoacid 1. Compound 20 is made following a hydrogenation of compound 19. Mono acid 20 reacts with corresponding amino esters in the presence of Aldrithiol-2 and triphenylphosphine to form compound 21.

Scheme 8

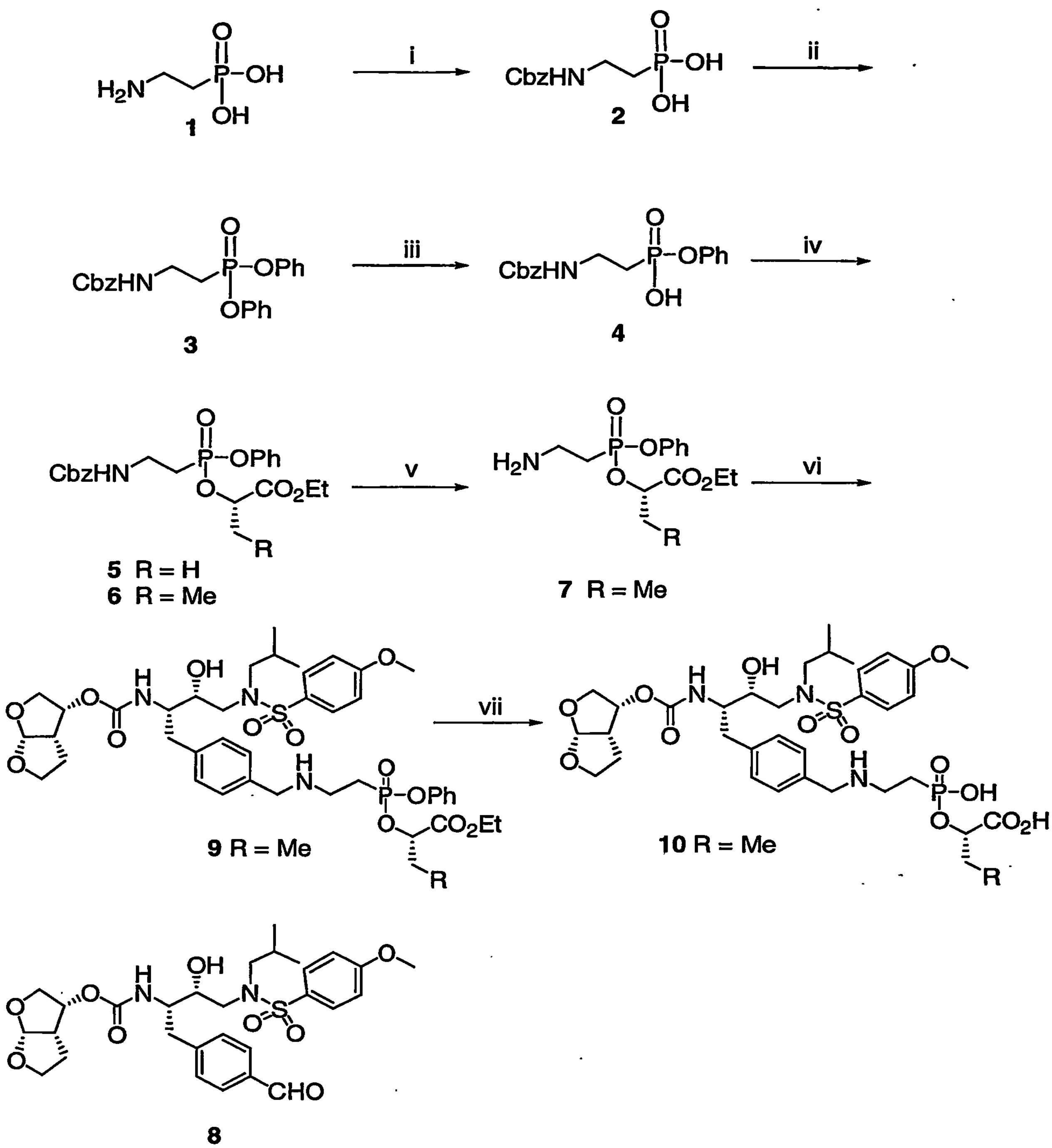


26 R = Me, Pr, i-Pr

I. a. $SOCl_2$/60 C; b. Alkyl (s)-lactate/Et_3N; II. H_2/10%Pd-C/EtOAc-HOAc; III. a. compound 25/$MgSO_4$;b. HOAc/$NaBH_3CN$

Monoacid 22 is treated with thionyl chloride at 60°C to form monochloridate, which reacts with corresponding alkyl (s)lactate to generate monolactate 23. Monolactate 23 is hydrogenated with 10%Pd-C in the presence of acetic acid to form amine 24. Aldehyde 25 reacts with amine 24 in the presence of $MgSO_4$ to form the intermediate imine, which is reduced with sodium cyanoborohydride to afford compound 26.

Scheme 1



Reagents and conditions: i. CbzCl, NaOH, tol/H_2O, 100%; ii. a. $SOCl_2$, DMF, tol, 65°C; b. PhOH, Et_3N, CH_2Cl_2, 71%; iii. aq. NaOH, CH_3CN, 79%; iv. a. $SOCl_2$, DMF, tol, 65°C; b. ethyl lactate, Et_3N, CH_2Cl_2, (**5**) 85%; 2-hydroxy butyric acid ethyl ester, Et_3N, CH_2Cl_2, (**6**) 75%; v. H_2, AcOH, 10% Pd/C, EtOH, 94%; vi. a. **7** + **8**, 1,2-DCE, $MgSO_4$; b. $NaBH_3CN$, AcOH, 50%; vii. pig liver esterase, 20% DMSO/PBS, 40°C, 25%.

Example 1

Compound 2: A 3L, 3-neck flask was equipped with a mechanical stirrer and addition funnel and charged with 2-aminoethyl phosphonic acid (60.0g, 480 mmol). 2N Sodium hydroxide (480 mL, 960 mmol) was added and flask cooled to 0°C. Benzyl chloroformate (102.4 g, 600 mmol) in toluene (160mL) was added dropwise with vigorous stirring. The reaction mixture was stirred at 0°C for 30 minutes, then at room temperature for 4 h. 2N sodium hydroxide (240 mL, 480 mmol) was added, followed by benzyl chloroformate (20.5 g, 120 mmol) and the reaction mixture was vigorously stirred for 12 h. The reaction mixture was washed with diethyl ether (3x). The aqueous layer was acidified to pH 2 with concentrated HCl to give a white precipitate. Ethyl acetate was added to the mixture and concentrated HCl (80 mL, 960 mmol) was added. The aqueous layer was extracted with ethyl acetate and combined organic layer was dried ($MgSO_4$) and concentrated to give a waxy, white solid (124 g, 479 mmol, 100%). ^1H NMR (300 MHz, CD_3OD): δ 7.45-7.30 (m, 5 H, Ar), 5.06 (d, J = 14.7 Hz, 2 H, CH_2Ph), 3.44-3.31 (m, 2 H, NCH_2CH_2), 2.03-1.91 (m, 2 H, CH_2CH_2P); ^{31}P NMR (121 MHz, CD_3OD): δ 26.3.

Example 2

Compound 3: To a mixture of compound 2 (50.0 g, 193 mmol) in toluene (1.0 L) was added DMF (1.0 mL) followed by thionyl chloride (56 mL, 768 mmol). The reaction mixture was heated at 65°C for 3-4 h under a stream of argon. The reaction mixture was cooled to room temperature and concentrated. Residual solvent was removed under high vacuum for 1 h. The residue was dissolved in CH_2Cl_2 (1.0 L) and cooled to 0°C. Triethylamine (161 mL, 1158 mmol) was added, followed by phenol (54.5 g, 579 mmol). The reaction mixture was warmed to room temperature overnight, then washed with 1.0N HCl, saturated $NaHCO_3$ solution, brine and dried ($MgSO_4$). Concentrated and purified (silica gel, 1:1 EtOAc/Hex) to give a pale yellow solid (56 g, 136 mmol, 71%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.40-7.10 (m, 15 H, Ar), 5.53 (br s, 1 H, NH), 5.11 (br s, 2 H, CH_2Ph), 3.72-3.60 (m, 2 H, NCH_2CH_2), 2.49-2.30 (m, 2 H, CH_2CH_2P); ^{31}P NMR (121 MHz, $CDCl_3$): δ 22.9.

Example 3

Compound 4: To a solution of compound 3 (64 g, 155.6 mmol) in acetonitrile (500 mL) at 0°C was added 2.0M sodium hydroxide. The reaction mixture was stirred at 0°C for 30 min, then at room temperature for 2.5 h. The reaction mixture was concentrated to 100 mL and diluted with H_2O (500 mL). The aqueous solution was washed with EtOAc (3 x 300 mL). The aqueous layer was acidified to pH 1 with concentrated HCl, producing a white precipitated. The mixture was extracted with EtOAc (4 x 300 mL) and combined organic layer was washed with brine and dried ($MgSO_4$). Concentration gave a solid, which was recrystallized from hot EtOAc (450 mL) to give a white solid (41.04 g, 122 mmol, 79%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.45-7.10 (m, 10 H, Ar), 5.09 (s, 2 H, CH_2Ph), 3.53-3.30 (m, 2 H, NCH_2CH_2), 2.25-2.10 (m, 2 H, CH_2CH_2P); ^{31}P NMR (121 MHz, CD_3OD): δ 24.5.

Example 4

Compound 5: To a mixture of compound 4 (28 g, 83 mmol) in toluene (500 mL) was added DMF (1.0 mL), followed by thionyl chloride (36.4 mL, 499 mmol). The mixture was heated at 65°C for 2 h providing a pale yellow solution. The reaction mixture was concentrated and dried for 45 min under high vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (350 mL) and cooled to 0°C. Triethylamine (45.3 mL, 332 mmol) was added slowly, followed by the dropwise addition of ethyl lactate (18.8 mL, 166 mmol). The reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with 1 N HCl, saturated $NaHCO_3$ solution, brine and dried ($MgSO_4$). Concentration and purification (silica gel, 1:5 to 1:0 EtOAc/Hex) gave a pale yellow oil (30.7 g, 71 mmol, 85%) as a mixture of diastereomers which were separated by HPLC (Dynamax reverse phase C–18 column, 60% acetonitrile/H_2O). More polar diastereomer: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40-7.10 (m, 10 H, Ar), 5.65 (s, 1 H, N*H*), 5.12 (s, 2 H, CH_2Ph), 5.10-5.00 (m, 1 H, OC*H*C) 4.17 (q, J = 6.9 Hz, 2 H, OCH_2CH_3), 3.62 (dt, J_1 = 20.4 Hz, J_2 = 6.0 Hz, 2 H, NCH_2CH_2), 2.25 (dt, J_1 = 18.0 Hz, J_2 = 6.0 Hz, 2 H, CH_2CH_2P), 1.60 (dd, J_1 = J_2= 6.9 Hz, 3 H, $CHCH_3$), 1.23 (t, J = 6.9 Hz, 3 H, OCH_2CH_3); ^{31}P NMR (121 MHz, $CDCl_3$): δ 26.2. Less polar diastereomer: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40-7.10 (m, 10 H, Ar), 5.87 (s, 1 H, N*H*), 5.13 (s, 2 H, CH_2Ph), 5.10-5.00 (dq, J_1 = J_2 = 6.9 Hz, 1 H, OC*H*C) 4.22 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 3.68 (dt, J_1 = 21.6 Hz, J_2 = 6.9 Hz, 2 H, NCH_2CH_2), 2.40-2.20 (m, 2 H, CH_2CH_2P), 1.49 (dd, J_1 = 70.2 Hz, J_2 = 6.9 Hz, 3 H, $CHCH_3$), 1.28 (t, J = 6.9 Hz, 3 H, OCH_2CH_3); ^{31}P NMR (121 MHz, $CDCl_3$): δ 28.3.

Example 5

Compound 6: 2-Hydroxy-butyric acid ethyl ester was prepared as follows: To a solution of L-2-aminobutyric acid (100g, 970 mmol) in 1.0 N H_2SO_4 (2 L) at 0°C was added $NaNO_2$ (111 g, 1610 mmol) in H_2O (400 mL) over 2 h. The reaction mixture was stirred at room temperature for 18h. Reaction mixture was extracted with EtOAc (4x) and combined organic layer was dried ($MgSO_4$) and concentrated to give a yellow solid (41.5 g). This solid was dissolved in absolute ethanol (500 mL) and concentrated HCl (3.27 mL, 39.9 mmol) was added. Reaction mixture was heated to 80°C. After 24 h, concentrated HCl (3 mL) was added and reaction continued for 24 h. Reaction mixture was concentrated and product was distilled to give a colorless oil (31 g, 235 mmol, 59%).

To a mixture of compound 4 (0.22 g, 0.63 mmol) in anhydrous acetonitrile (3.0 mL) was added thionyl chloride (0.184 mL, 2.52 mmol). The mixture was heated at 65°C for 1.5 h providing a pale yellow solution. The reaction mixture was concentrated and dried for 45 min under high vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (3.3 mL) and cooled to 0°C. Triethylamine (0.26 mL, 1.89 mmol) was added slowly, followed by the dropwise addition of 2-hydroxy-butyric acid ethyl ester (0.167 mL, 1.26 mmol). The reaction mixture was stirred at 0°C for 5 min, then warmed to room temperature overnight. The reaction mixture was concentrated, dissolved in EtOAc and washed with 1.0 N HCl, saturated $NaHCO_3$ solution, brine and dried ($MgSO_4$). Concentration and purification (silica gel, 3:2 EtOAc/Hex) gave a pale yellow oil (0.21 g, 0.47 mmol, 75%). For major diastereomer, ^1H NMR (300 MHz, $CDCl_3$): δ 7.35-7.10 (m, 10 H, Ar), 5.91 (s, 1 H, N*H*)), 5.12 (s, 2 H, CH_2Ph), 4.94-4.83 (m, 1 H, OC*H*C), 4.27-4.12 (m, 2 H, OCH_2CH_3), 3.80-3.50 (m, 2 H, NCH_2CH_2), 2.39-2.19 (m, 2 H, CH_2CH_2P), 1.82-1.71 (m, 2 H, $CHCH_2CH_3$), 1.30-1.195 (m, 3 H, OCH_2CH_3), 0.81 (t, J = 7.5 Hz, 3 H, $CHCH_2CH_3$); ^{31}P NMR (120 MHz, $CDCl_3$): δ 28.3. For minor diastereomer, ^1H NMR (300 MHz, $CDCl_3$): δ 7.35-7.10 (m, 10 H, Ar), 5.74 (s, 1 H, N*H*)), 5.11 (s, 2 H, CH_2Ph), 4.98-4.94 (m, 1 H, OC*H*C), 4.27-4.12 (m, 2 H, OCH_2CH_3), 3.80-3.50 (m, 2 H, NCH_2CH_2), 2.39-2.19 (m, 2 H, CH_2CH_2P), 1.98-1.82 (m, 2 H, $CHCH_2CH_3$), 1.30-1.195 (m, 3 H, OCH_2CH_3), 1.00 (t, J = 7.5 Hz, 3 H, $CHCH_2CH_3$); ^{31}P NMR (121 MHz, $CDCl_3$): δ 26.2.

Example 6

Compound 7: A mixture of compound **6**, (0.53 g, 1.18 mmol) acetic acid (0.135 mL, 2.36 mmol) and 10% palladium on activated carbon (0.08 g) in absolute ethanol (12 mL) was stirred under a hydrogen atmosphere (1 atm) for 3 h. Reaction mixture was filtered through Celite, concentrated, and resubjected to identical reaction conditions. After 2 h, Celite was added to the reaction mixture and mixture was stirred for 2 min, then filtered through a pad of Celite and concentrated. Dried under high vacuum to give the diasteromeric acetate salt as a oil (0.42 g, 1.11 mmol, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40-7.10 (m, 5 H, Ar), 5.00-4.80 (m, 1 H, OC*H*C), 4.28-4.10 (m, 2 H, OCH_2CH_2), 3.32-3.14 (m, 2 H, NCH_2CH_2), 2.45-2.22 (m, 2 H, CH_2CH_2P), 1.97 (s, 3 H, Ac), 1.97-1.70 (m, 2 H, $CHCH_2CH_3$), 1.30-1.18 (m, 3 H, OCH_2CH_3), 1.00 (t, J = 7.5 Hz, 1 H, $CHCH_2CH_3$), 0.80 (t, J = 7.5 Hz, 2 H, $CHCH_2CH_3$); ^{31}P NMR (121 MHz, $CDCl_3$): δ 27.6 (major, 1.85), 26.0 (minor, 1.01).

Example 7

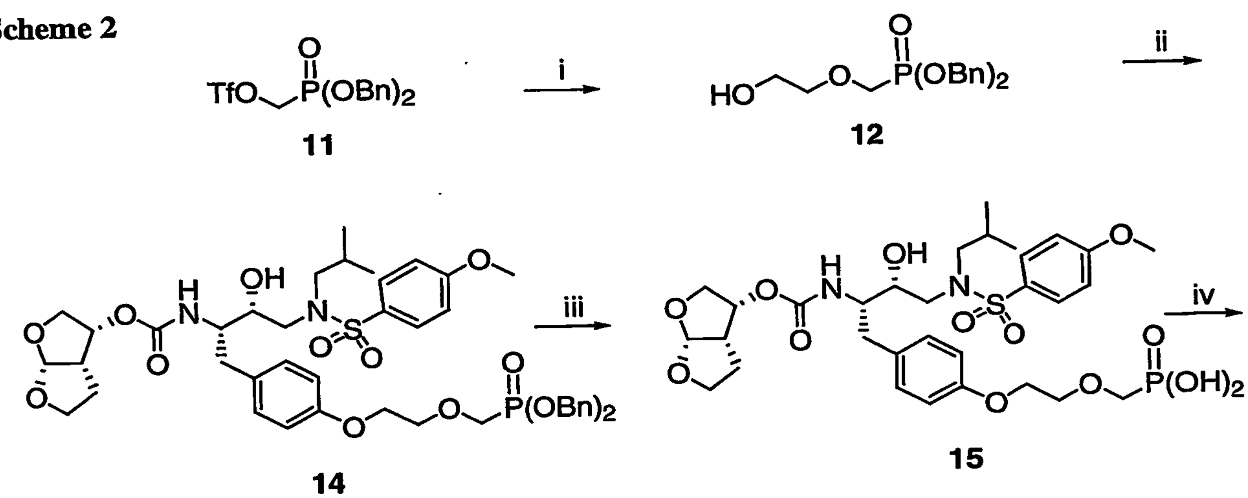
Compound 9: A solution of aldehyde **8** (0.596 g, 1.01 mmol) and compound **7** (0.42 g, 1.11 mmol) were stirred together in 1,2-dichloroethane (4.0 mL) in the presence of $MgSO_4$ for 3 h. Acetic acid (0.231 mL, 4.04 mmol) and sodium cyanoborohydride (0.127 g, 2.02 mmol) were added and reaction mixture was stirred for 50 min at room temperature. Reaction mixture was quenched with saturated $NaHCO_3$ solution, diluted with EtOAc, and vigorously stirred for 5 min. Brine was added and extracted with EtOAc (2x). Combined organic layer was dried ($MgSO_4$) concentrated and purified (silica gel, EtOAc, then 10% EtOH/EtOAc) to give a colorless foam. Acetonitrile (4 mL) and trifluoroacetic acid (0.06 mL) were added and concentrated to a volume of 1 mL. H_2O (10 mL) was added and lyophilized to give the TFA salt as a white powder (0.51 g, 0.508 mmol, 50%). ^{1}H NMR (300 MHz, CD_3CN): δ 7.79 (d, J = 8.4 Hz, 2 H, ($SO_2C(CH)_2$), 7.43-7.20 (m, 9 H, Ar), 7.10 (d, J = 8.4 Hz, 2 H, $(CH)_2COCH_3$), 5.85 (d, J = 8.4 Hz, 1 H, N*H*), 5.55 (d, J = 4.5 Hz, 1 H, OC*H*O), 5.00-4.75 (m, 2 H, $CH_2CHOC(O)$, POC*H*C), 4.39-4.05 (m, 2 H, $PhCH_2N$, OCH_2CH_3), 3.89 (s, 3 H, OCH_3), 3.88-3.30 (m, 9H), 3.15-2.84 (m, 5 H), 2.65-2.42 (m, 3 H), 2.10-1.68 (m, 5 H), 1.65-1.15 (m, 5 H), 1.05-0.79 (m, 9 H); ^{31}P NMR (121 MHz, CD_3CN): δ 24.8 (major, 1.85), 23.1 (minor, 1.01).

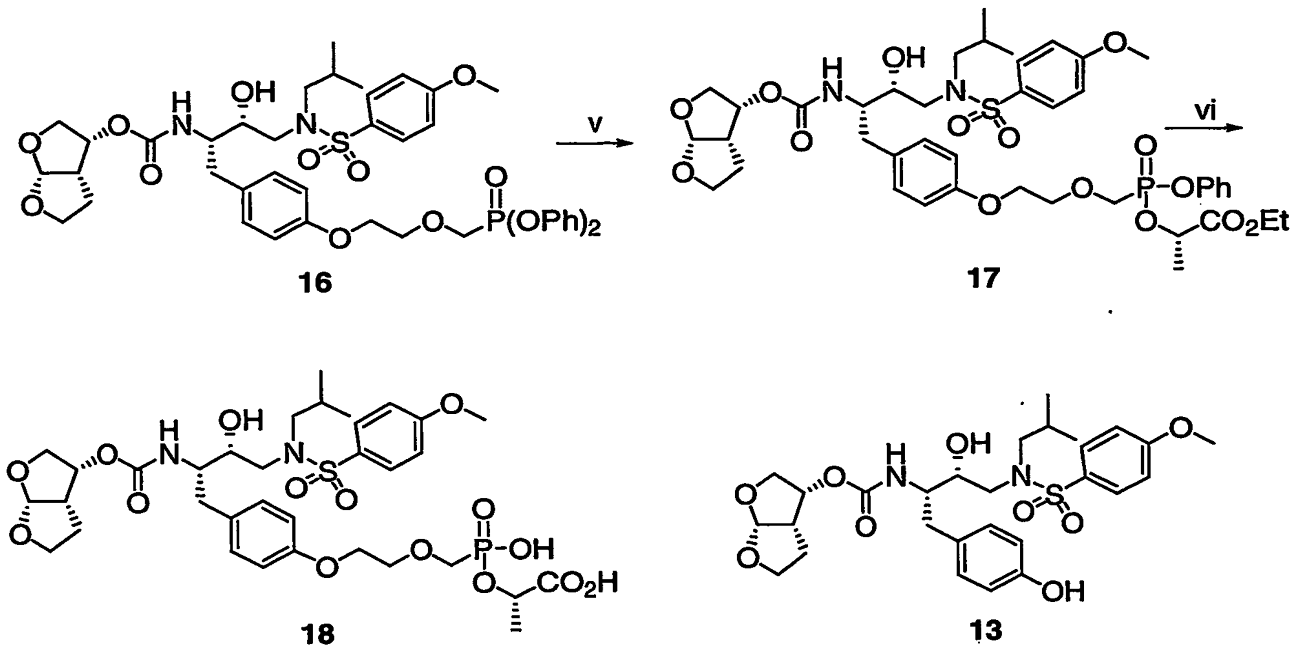
Example 8

Compound 10: Compound **9** (0.041 g, 0.041 mmol) was dissolved in DMSO (1.9 mL) and to this solution was added phosphate buffered saline, pH 7.4 (10 mL) and pig liver esterase

(Sigma, 0.2 mL). Reaction mixture was stirred for 24 h at 40°C. After 24 h, additional esterase (0.2 mL) was added and reaction was continued for 24 h. Reaction mixture was concentrated, resuspended in methanol and filtered. Filtrate was concentrated and purified by reverse phase chromatography to give a white powder after lyophilization (8 mg, 0.010 mmol, 25%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.78 (d, J = 8.9 Hz, 2 H, ($SO_2C(C\mathit{H})_2$), 7.43-7.35 (m, 4 H, Ar), 7.11 (d, J = 8.9 Hz, 2 H, ($(C\mathit{H})_2COCH_3$), 5.62 (d, J = 5.2 Hz, 1 H, OC*H*O), 4.96-4.77 (m, 2 H, $CH_2C\mathit{H}OC(O)$, POC*H*C), 4.21 (br s, 2 H, $PhC\mathit{H}_2N$), 3.97-3.70 (m, 6 H), 3.90 (s, 3 H, $OC\mathit{H}_3$), 3.50-3.30 (m, 3 H), 3.26-3.02 (m, 2 H), 2.94-2.58 (m, 4 H), 2.09-1.78 (m, 5 H), 1.63-1.52 (m, 2 H), 1.05-0.97 (m, 3 H); 0.94 (d, J = 6.7 Hz, 3 H), 0.88 (d, J = 6.7 Hz, 3 H); ^{31}P NMR (121 MHz, CD_3OD): δ 20.8.

Scheme 2





16 17 18 13

Reagents and conditions: i. ethylene glycol, $Mg(OtBu)_2$, DMF, 48%; ii. a. Tf_2O, 2,6-lutidine, CH_2Cl_2, -78°C; b. **13**, $CsCO_3$, CH_3CN, 0°C to room temperature, 65%; iii. H_2, Pd/C, EtOH, 107%; iv. DCC, PhOH, pyr, 70°C, 31%; v. a. NaOH, CH_3CN, 0°C; b. DCC, ethyl lactate, pyr, 70°C, 52%; vi. CH_3CN, DMSO, PBS, porcine liver esterase, 38°C, 69%.

Example 9

Compound 12: To a solution of compound **11** (4.10 g, 9.66 mmol) and anhydrous ethylene glycol (5.39 mL, 96.6 mmol) in anhydrous DMF (30 mL) at 0°C was added powdered magnesium *tert*-butoxide (2.05 g, 12.02 mmol). The reaction mixture was stirred at 0°C for 1.5 h, then concentrated. The residue was partitioned between EtOAc and H_2O and washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine. Organic layer dried ($MgSO_4$), concentrated and purified (silica gel, 4% MeOH/CH_2Cl_2) to give a colorless oil (1.55 g, 48%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.37 (s, 10 H, Ar), 5.40-5.05 (m, 4 H, CH_2Ph), 3.84 (d, J = 8.1 Hz, 2 H, PCH_2O), 3.70-3.60 (m, 4 H, OCH_2CH_2O, OCH_2CH_2O); ^{31}P NMR (121 MHz, $CDCl_3$): δ 22.7.

Example 10

Compound 14: To a solution of compound **12** (0.75 g, 2.23 mmol) and 2,6-lutidine (0.78 mL, 6.69 mmol) in CH_2Cl_2 (20 mL) at –78°C was added trifluoromethanesulfonic anhydride (0.45

mL, 2.68 mmol). The reaction mixture was stirred at –78°C for 40 min, then diluted with CH_2Cl_2 and washed with 1 N HCl, saturated $NaHCO_3$ and dried ($MgSO_4$). Concentration gave a yellow oil that was dissolved in anhydrous acetonitrile (20 mL). Phenol **13** (1.00 g, 1.73 mmol) was added to the solution, which was cooled to 0°C. Cesium carbonate (0.619 g, 1.90 mmol) was added and reaction mixture was stirred at 0°C for 2 h, then at room temperature for 1.5 h. Additional cesium carbonate (0.200 g, 0.61 mmol) was added and reaction was continued for 1.5 h, then filtered. Concentration of the filtrate and purification (silica gel, 3% MeOH/CH_2Cl_2) gave a yellow gum (1.005 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, J = 8.7 Hz, 2 H, $SO_2C(CH)_2$), 7.34 (s, 10 H, $PhCH_2O$), 7.11 (d, J = 8.1Hz, 2 H, $CH_2C(CH)_2(CH)_2$), 6.98 (d, J = 8.7 Hz, 2 H, $(CH)_2COCH_3$), 6.78 (d, J = 8.7 Hz, 2 H, $(CH)_2COCH_2$), 5.62 (d, J = 5.4 Hz, 1 H, OC*H*O), 5.16-4.97 (m, 6 H), 4.05-3.65 (m, 12 H), 3.86 (s, 3 H, OCH_3), 3.19-2.66 (m, 7 H), 1.95-1.46 (m, 3 H), 0.92 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$), 0.88 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$); ^{31}P NMR (121 MHz, $CDCl_3$): δ 21.9.

Example 11

Compound 15: A mixture of compound **14** (0.410 g, 0.457 mmol) and 10% palladium on carbon (0.066 g) in ethanol (5.0 mL) was stirred under a hydrogen atmosphere (1 atm) for 16 h. Celite was added and the mixture was stirred for 5 min, then filtered through Celite and concentrated to give a foam (0.350 g, 107%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.76 (d, J = 8.7 Hz, 2 H, $SO_2C(CH)_2$), 7.15 (d, J = 8.4Hz, 2 H, $CH_2C(CH)_2(CH)_2$), 7.08 (d, J = 8.4 Hz, 2 H, $(CH)_2COCH_3$), 6.82 (d, J = 8.4 Hz, 2 H, $(CH)_2COCH_2$), 5.59 (d, J = 5.4 Hz, 1 H, OC*H*O), 5.16-4.97 (masked by CD_3OH, 1 H), 4.09-4.02 (m, 2 H), 3.99-3.82 (m, 10 H), 3.88 (s, 3 H, OCH_3), 3.52-3.32 (m, 1 H), 3.21-2.75 (m, 5 H), 2.55-2.40 (m, 1 H), 2.10-1.95 (m, 1 H), 1.75-1.25 (m, 2 H), 0.93 (d, J = 6.3 Hz, 3 H, $CH(CH_3)_2$), 0.88 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$); ^{31}P NMR (121 MHz, CD_3OD): δ 19.5.

Example 12

Compound 16: Compound **15** (0.350 g, 0.488 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL), each time filling with N_2. Residue was dissolved in anhydrous pyridine (2.5 mL) and phenol (0.459 g, 4.88 mmol) was added. This solution was heated to 70°C, then 1,3-dicyclohexylcarbodiimide (0.403 g, 1.93 mmol) was added and reaction mixture was heated at 70°C for 7 h. Reaction mixture was concentrated, coevaporated with toluene and

residue obtained was diluted with EtOAc, precipitating 1,3-dicyclohexylurea. The mixture was filtered and filtrate concentrated and residue obtained was purified (silica gel, 2% $MeOH/CH_2Cl_2$, then another column 75% EtOAc/Hex) to give a clear oil (0.1324 g, 31%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, J = 8.7 Hz, 2 H, $SO_2C(CH)_2$), 7.41-7.18 (m, 10 H, Ar), 7.14 (d, J = 8.4Hz, 2 H, $CH_2C(CH)_2(CH)_2$), 6.99 (d, J = 9.0 Hz, 2 H, $(CH)_2COCH_3$), 6.83 (d, J = 8.4 Hz, 2 H, $(CH)_2COCH_2$), 5.64 (d, J = 5.1 Hz, 1 H, OCHO), 5.16-4.92 (m, 2 H), 4.32-3.62 (m, 12 H), 3.87 (s, 3 H, OCH_3), 3.22-2.73 (m, 7 H), 1.95-1.75 (m, 3 H), 0.93 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$), 0.88 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$); ^{31}P NMR (121 MHz, $CDCl_3$): δ 14.3.

Example 13

Compound 17: To a solution of compound **16** (0.132 g, 0.152 mmol) in acetonitrile (1.5 mL) at 0°C was added 1.0 M NaOH (0.38 mL, 0.381 mmol). Reaction mixture was stirred for 2 h at 0°C, then Dowex 50 (H+) resin was added until pH = 1. The resin was removed by filtration and the filtrate was concentrated and washed with EtOAc/Hex (1:2, 25 mL), then dried under high vacuum to give a clear film (0.103 g, 85%). This film was coevaporated with anhydrous pyridine (3 x 5 mL), filling with N_2. The residue was dissolved in anhydrous pyridine (1 mL) and ethyl lactate (0.15 mL, 1.30 mmol) was added and reaction mixture was heated at 70°C. After 5 min, 1,3-dicyclohexylcarbodiimide (0.107 g, 0.520 mmol) was added and reaction mixture was stirred at 70°C for 2.5 h. Additional 1,3-dicyclohexylcarbodiimide (0.055 g, 0.270 mmol) was added and reaction continued for another 1.5 h. Reaction mixture was concentrated and coevaporated with toluene and diluted with EtOAc, precipitating 1,3-dicyclohexylurea. The mixture was filtered and filtrate concentrated and residue obtained was purified (silica gel, 80 to 100% EtOAc/Hex) to give a white foam (0.0607 g, 52%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, J = 8.7 Hz, 2 H, $SO_2C(CH)_2$), 7.39-7.16 (m, 5 H, Ar), 7.13 (d, J = 8.1Hz, 2 H, $CH_2C(CH)_2(CH)_2$), 6.99 (d, J = 9.0 Hz, 2 H, $(CH)_2COCH_3$), 6.82 (d, J = 8.4 Hz, 2 H, $(CH)_2COCH_2$), 5.64 (d, J = 5.1 Hz, 1 H, OCHO), 5.16-4.92 (m, 3 H), 4.35-3.65 (m, 14 H), 3.87 (s, 3 H, OCH_3), 3.22-2.73 (m, 7 H), 1.95-1.80 (m, 3 H), 1.59 (d, J = 6.9Hz, 1.5 H, $CCHCH_3$), 1.47 (d, J = 7.2 Hz, 1.5 H, $CCHCH_3$), 1.37-1.18 (m, 3 H), 0.92 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$), 0.88 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$); ^{31}P NMR (121 MHz, $CDCl_3$): δ 19.2, 17.2.

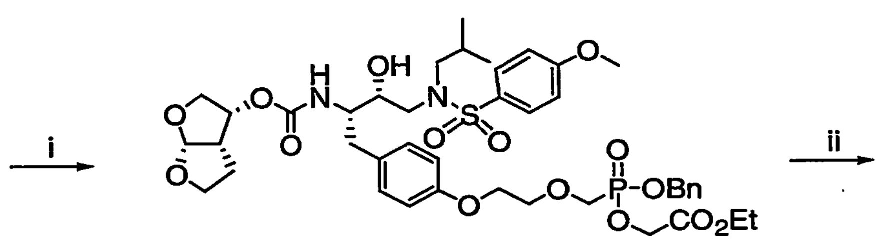
Example 14

Compound 18: Compound **17** (11.5 mg, 0.013 mmol) was dissolved in DMSO (0.14 mL) and acetonitrile (0.29 mL). PBS (pH 7.4, 1.43 mL) was added slowly with stirring. Porcine liver esterase (Sigma, 0.1 mL) was added and reaction mixture was gently stirred at 38°C. After 24 h, additional porcine liver esterase (0.1 mL) and DMSO (0.14 mL) were added and reaction mixture stirred for 48 h at 38°C. Reaction mixture concentrated and methanol was added to precipitate the enzyme. The mixture was filtered, concentrated and purified by reverse phase chromatography to give a white powder after lyophilization (7.1 mg, 69%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.76 (d, J = 8.7 Hz, 2 H, $SO_2C(CH)_2$), 7.15 (d, J = 8.4 Hz, 2 H, $CH_2C(CH)_2(CH)_2$), 7.08 (d, J = 9.0 Hz, 2 H, $(CH)_2COCH_3$), 6.83 (d, J = 8.7 Hz, 2 H, $(CH)_2COCH_2$), 5.59 (d, J = 5.1 Hz, 1 H, OC*H*O), 5.16-4.90 (masked by CD_3OH, 2 H), 4.19-3.65 (m, 12 H), 3.88 (s, 3 H, OCH_3), 3.50-3.27 (m, 1 H), 3.20-2.78 (m, 5 H), 2.55-2.40 (m, 1 H), 2.05-1.90 (m, 1 H), 1.75-1.30 (m, 2 H), 1.53 (d, J = 6.6 Hz, 3 H, $CCHCH_3$), 0.93 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$), 0.88 (d, J = 6.6 Hz, 3 H, $CH(CH_3)_2$); ^{31}P NMR (121 MHz, CD_3OD): δ 16.7.

Alternatively, compound **17** was prepared as described below (Scheme 3).

Scheme 3

14



19

17

Reagents and conditions: i. a. **14**, DABCO, tol, reflux, b. ethyl lactate, PyBOP, DIPEA, DMF, 59%; ii. a. H_2, Pd/C, EtOH; b. PhOH, PyBOP, DIPEA, DMF, 35%.

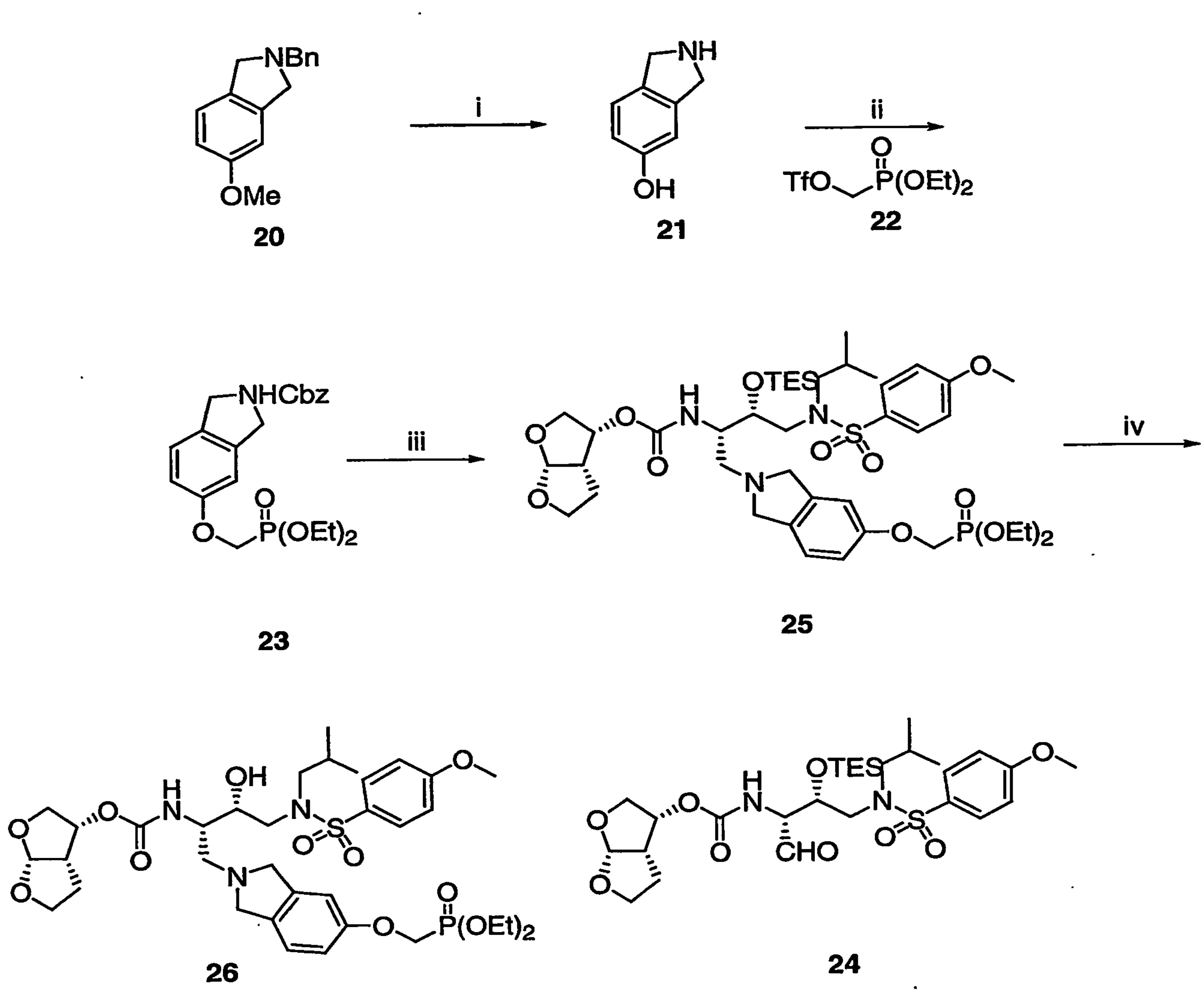
Example 15

Compound 19: To a solution of compound **14** (0.945 g, 1.05 mmol) in anhydrous toluene (10.0 mL) was added 1,4-diazobicyclo[2.2.2] octane (0.130 g, 1.16 mmol) and reaction mixture was refluxed for 2 h. After cooling to room temperature, reaction mixture was diluted with EtOAc and washed with 1.0 N HCl and dried ($MgSO_4$). Concentration gave a white foam (0.785 g, 93%). Residue was dissolved in anhydrous DMF (10.0 mL) and to this solution was added ethyl (S)-lactate (0.23 mL, 2.00 mmol) and diisopropylethylamine (0.70 mL, 4.00 mmol), followed by benzotriazol-1-yloxytripyrroldinophosphonium hexafluorophosphate (1.041 g, 2.00 mmol). Reaction mixture was stirred for 20 h, then concentrated and residue was dissolved in EtOAc and washed with 1.0 N HCl, saturated $NaHCO_3$, brine and dried ($MgSO_4$). Concentration and purification (silica gel, 2 % $MeOH/CH_2Cl_2$) gave an off-white foam (0.520 g, 59%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.72 (d, J = 7.5 Hz, 2 H, $SO_2C(CH)_2$), 7.50-7.27 (m, 4 H, Ar), 7.12 (d, J = 8.1Hz, 2 H, $CH_2C(CH)_2(CH)_2$), 7.00 (d, J = 6.6 Hz, 2 H, $(CH)_2COCH_3$), 6.81 (d, J = 8.4 Hz, 2 H, $(CH)_2COCH_2$), 5.64 (d, J = 5.1 Hz, 1 H, OCHO), 5.37-4.90 (m, 5 H), 4.35-3.65 (m, 14 H), 3.88 (s, 3 H, OCH_3), 3.24-2.70 (m, 7 H), 1.90-1.70 (m, 3 H), 1.54 (d, J = 6.9Hz, 1.5 H, $CCHCH_3$), 1.47 (d, J = 6.9 Hz, 1.5 H, $CCHCH_3$), 1.37-1.22 (m, 3 H), 0.93 (d, J = 6.3 Hz, 3 H, $CH(CH_3)_2$), 0.89 (d, J = 6.0 Hz, 3 H, $CH(CH_3)_2$); ^{31}P NMR (121 MHz, $CDCl_3$): δ 22.3, 21.2.

Example 16

Compound 17: A mixture of compound **19** (0.520 g, 0.573 mmol) and 10% palladium on carbon (0.055 g) in ethanol (10 mL) was stirred under a hydrogen atmosphere (1 atm) for 2 h. Celite was added to the reaction mixture and stirred for 5 min, then mixture was filtered through Celite and concentrated to give a white foam (0.4649 g, 99%). Residue was dissolved in anhydrous DMF (5.0 mL) and to this solution was added phenol (0.097 g, 1.03 mmol), diisopropylethylamine (0.36 mL, 2.06 mmol) followed by benzotriazol-1-yloxytripyrroldinophosphonium hexafluorophosphate (0.536 g, 1.03 mmol). Reaction mixture was stirred for 20 h, then concentrated and residue was dissolved in EtOAc and washed with 1 N HCl, H_2O, sat. $NaHCO_3$, brine and dried ($MgSO_4$). Concentration and purification (silica gel, 2 % $MeOH/CH_2Cl_2$) gave a white foam (0.180 g, 35%).

Scheme 4



Reagents and conditions: i. a. 48% HBr, 120°C, 65%; b. H_2, $Pd(OH)_2$, EtOH, 100%;
ii. CbzCl, NaOH, tol/H_2O, 0°C to rt, 43%; b. **22**, $CsCO_3$, CH_3CN, 99%;
iii. a. H_2, Pd/C, AcOH, EtOAc/EtOH, 95%; b. **24**, $NaBH(OAc)_3$, 1,2-DCE, 21%;
iv, 4% HF/CH_3CN, 62%.

Example 17

Compound 21: Compound **20** (11.5 g, 48.1 mmol) in 48% HBr (150 mL) was heated at 120°C for 4 h, then cooled to room temperature and diluted with EtOAc. Mixture was neutralized with saturated $NaHCO_3$ solution and solid $NaHCO_3$ and extracted with EtOAc containing MeOH. Organic layer dried ($MgSO_4$), concentrated, and purified (silica gel, 1:2

EtOAc/Hex with 1% MeOH) to give a brown solid (7.0 g, 65%). The resulting compound (7.0 g, 31.1 mmol) and 10% palladium hydroxide (2.1 g) in EtOH (310 mL) was stirred under a hydrogen atmosphere for 1 d, then filtered through Celite and concentrated to give an off-white solid (4.42 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.01 (d, J = 7.8 Hz, 1 H, Ar), 6.64 (s, 1 H, Ar), 6.61 (d, J = 8.1 Hz, 2 H, Ar), 4.07 (s, 2 H, ArCH_2N), 4.05 (s, 2 H, ArCH_2N).

Example 18

Compound 22: To a solution of compound **21** (4.42 g, 32.7 mmol) in 1.0 M NaOH (98 mL, 98.25 mmol) at 0°C was added dropwise benzyl chloroformate (7.00 mL, 49.13 mmol) in toluene (7 mL). After addition was complete, reaction mixture was stirred overnight at room temperature. Reaction mixture was diluted with EtOAc and extracted with EtOAc (3x). Combined organic layer was dried ($MgSO_4$), concentrated and purified (silica gel, 2% MeOH/CH_2Cl_2) to give a white solid (3.786 g, 43%). The resulting compound (0.6546 g, 2.43 mmol) was dissolved in anhydrous acetonitrile (10 mL), and compound **23** (0.782 g, 2.92 mmol) was added, followed by cesium carbonate (1.583 g, 4.86 mmol). Reaction mixture was stirred for 2h at room temperature, then filtered, concentrated, and purified (3% MeOH/CH_2Cl_2) to give a brownish oil (1.01 g, 99%).

Example 19

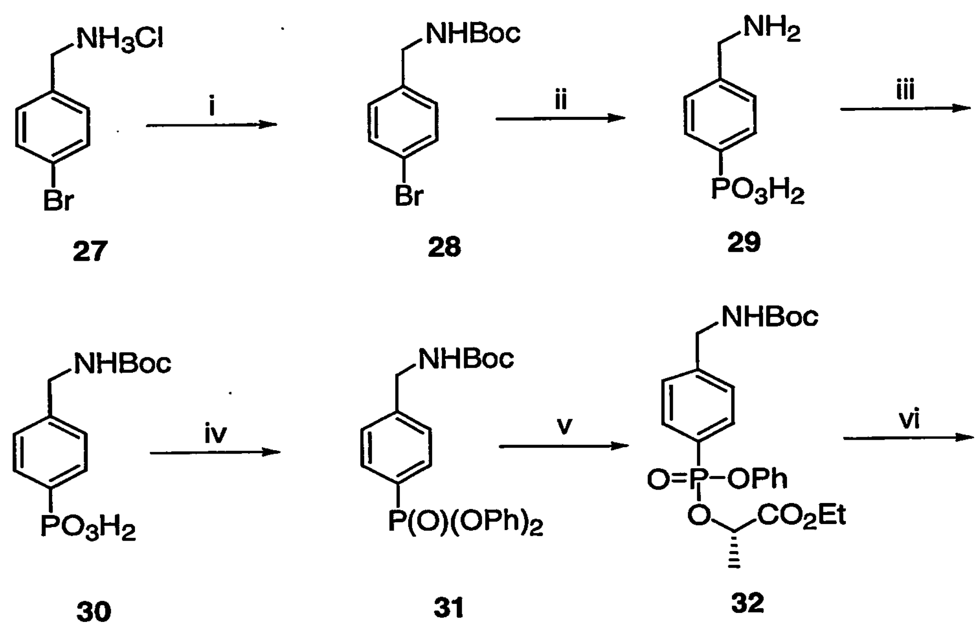
Compound 25: To a solution of compound **22** (0.100 g, 0.238 mmol) in EtOAc/EtOH (2 mL, 1:1) was added acetic acid (14 μL, 0.238 mmol) and 10% palladium on carbon (0.020 g) and the mixture was stirred under a hydrogen atmosphere for 2 h. Celite was added to the reaction mixture and stirred for 5 min, then filtered through Celite. Concentration and drying under high vacuum gave a reddish film (0.0777 g, 95%). The resulting amine (0.0777g, 0.225 mmol) and aldehyde **24** (0.126 g, 0.205 mmol) in 1,2-dichloroethane (1.2 mL) were stirred for 5 min at 0°C, then sodium triacetoxyborohydride (0.0608 g, 0.287 mmol) was added. Reaction mixture was stirred for 1 h at 0°C, then quenched with saturated $NaHCO_3$ solution and brine. Extracted with EtOAc, the organic layer was dried ($MgSO_4$), concentrated and purified (silica gel, 2% MeOH/CH_2Cl_2) to give a brown foam (38.7 mg, 21%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J = 8.7 Hz, 2 H, Ar), 7.09 (d, J = 8.7 Hz, 1 H, Ar), 7.05-6.72 (m, 4 H, Ar), 5.71 (d, J = 5.1 Hz, 1 H), 5.22-5.07 (m, 2 H), 4.22-4.17 (m, 7 H),

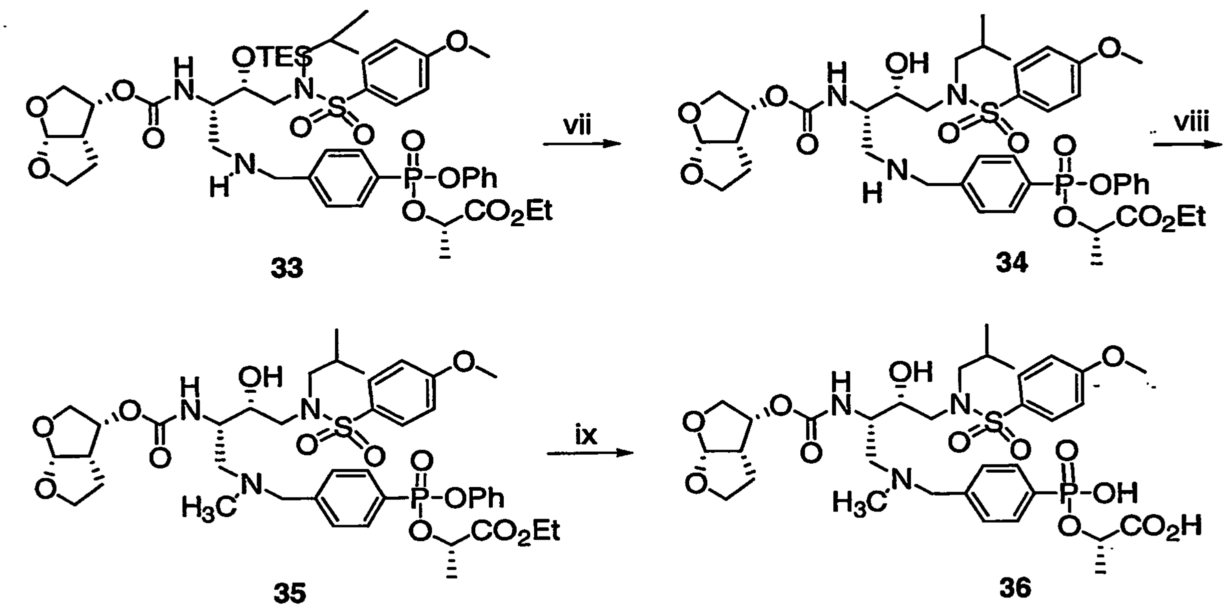
4.16-3.69 (m, 9 H), 3.82 (s, 3 H), 3.25-2.51 (m, 7 H), 2.22-1.70 (m, 3 H), 1.37 (t, J = 6.9 Hz, 6 H), 1.10-0.58 (m, 21 H); ^{31}P NMR (121 MHz, $CDCl_3$): δ 19.5.

Example 20

Compound 26: To a solution of compound 25 (38.7 mg, 0.0438 mmol) in acetonitrile (0.5 mL) at 0°C was added 48% HF (0.02 mL). The reaction mixture was stirred at room temperature for 2 h, then quenched with saturated $NaHCO_3$ solution and extracted with EtOAc. Organic layer was separated, dried ($MgSO_4$), concentrated and purified (silica gel, 3 to 5% MeOH/CH_2Cl_2) to give a red film (21.2 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.7 Hz, 2 H, Ar), 7.10 (d, J = 8.7 Hz, 1 H, Ar), 6.97 (d, J = 8.70 Hz, 2 H), 6.90-6.76 (m, 2 H), 5.72 (d, J = 5.1 Hz, 1 H), 5.41 (d, J = 9.0 Hz, 1 H), 5.15 (q, J = 6.6 Hz, 1 H), 4.38-4.17 (m, 7 H), 4.16-3.65 (m, 9 H), 3.87 (s, 3 H), 3.20-2.82 (m, 7 H), 2.75-1.79 (m, 3 H), 1.37 (t, J = 6.9 Hz, 6 H), 0.90 (d, J = 6.6 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 3 H); ^{31}P NMR (121 MHz, $CDCl_3$): δ 19.3.

Scheme 5





Reagents and conditions: i. Boc_2O, NaOH, H_2O, 96%;
ii. a. $HP(OEt)_2$, Et_3N, $(PPh_3)_4Pd$, 90°C, b. TMSBr, CH_3CN, 65%;
iii. Boc_2O, NaOH, THF/H_2O, 89%; iv. PhOH, DCC, pyr, 70°C, 71%;
v. a. NaOH, CH_3CN, 94%; b. Et lactate, DCC, pyr, 70°C, 80%; vi. a. TFA, CH_2Cl_2;
b. **24**, AcOH, $NaBH_3CN$, EtOH, 33%; vii. 4% HF/CH_3CN, 88%;
viii. HCHO, AcOH, $NaBH_3CN$, EtOH, 67%;
ix. CH_3CN, DMSO, PBS, porcine liver esterase, 38°C, 21%.

Example 21

Compound 28: To a mixture of 4-bromobenzylamine hydrochloride (15.23 g, 68.4 mmol) in H_2O (300 mL) was added sodium hydroxide (8.21 g, 205.2 mmol), followed by di-*tert*-butyl dicarbonate (16.45g, 75.3 mmol). Reaction mixture was vigorously stirred for 18 h, then diluted with EtOAc (500 mL). Organic layer separated and aqueous layer extracted with EtOAc (200 mL). Combined organic layer was dried ($MgSO_4$), concentrated and dried under high vacuum to give a white solid (18.7 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (d, J = 8.4 Hz, 2 H), 7.12 (d, J = 8.3 Hz, 2 H), 4.82 (s, 1 H, N*H*), 4.22 (d, J = 6.1 Hz, 2 H), 1.41 (s, 9 H).

Example 22

Compound 29: Compound **28** (5.00 g, 17.47 mmol) was coevaporated with toluene. Diethyl phosphite (11.3 mL, 87.36 mmol) was added and mixture was coevaporated with toluene

(2x). Triethylamine (24.0 mL, 174.7 mmol) was added and mixture was purged with argon for 10 min, then tetrakis(triphenylphosphine) palladium(0) (4.00 g, 3.49 mmol) was added. Reaction mixture was refluxed for 18 h, cooled, concentrated and diluted with EtOAc. Washed with 0.5 N HCl, 0.5 M NaOH, H_2O, brine and dried ($MgSO_4$). Concentrated and purification (silica gel, 70% EtOAc/Hex) gave an impure reaction product as a yellow oil (6.0 g). This material (6.0 g) was dissolved in anhydrous acetonitrile (30 mL) and cooled to 0°C. Bromotrimethylsilane (11.5 mL, 87.4 mmol) was added and reaction mixture was warmed to room temperature over 15 h. Reaction mixture was concentrated, dissolved in MeOH (50 mL) and stirred for 1.5 h. H_2O (1 mL) was added and mixture stirred for 2 h. Concentrated to dryness and dried under high vacuum, then triturated with Et_2O containing 2% MeOH to give a white solid (3.06 g, 65 %). ^1H NMR (300 MHz, D_2O): δ 7.67 (dd, J = 12.9, 7.6 Hz, 2 H), 7.45-7.35 (m, 2 H), 4.10 (s, 2 H); ^{31}P NMR (121 MHz, D_2O): δ 12.1.

Example 23

Compound 30: Compound **29** (4.78 g, 17.84 mmol) was dissolved in H_2O (95 mL) containing sodium hydroxide (3.57 g, 89.20 mmol). Di-*tert*-butyl dicarbonate (7.63 g, 34.94 mmol) was added, followed by THF (25 mL). The clear reaction mixture was stirred overnight at room temperature then concentrated to ~100 mL. Washed with EtOAc and acidified to pH 1 with 1 N HCl and extracted with EtOAc (7x). Combined organic layer was dried ($MgSO_4$), concentrated and dried under high vacuum. Trituration with Et_2O gave a white powder (4.56 g, 89%). ^1H NMR (300 MHz, CD_3OD): δ 7.85-7.71 (m, 2 H), 7.39-7.30 (m, 2 H), 4.26 (s, 2 H), 1.46 (s, 9 H); ^{31}P NMR (121 MHz, CD_3OD): δ 16.3.

Example 24

Compound 31: Compound **30** (2.96 g, 10.32 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL). To this residue was added phenol (9.71 g, 103.2 mmol) and mixture was coevaporated with anhydrous pyridine (2 x 10 mL). Pyridine (50 mL) was added and solution heated to 70°C. After 5 min, 1,3-dicyclohexylcarbodiimide (8.51 g, 41.26 mmol) was added and resulting mixture was stirred for 8 h at 70°C. Reaction mixture was cooled and concentrated and coevaporated with toluene. Residue obtained was diluted with EtOAc and the resulting precipitate was removed by filtration. The filtrate was concentrated and purified (silica gel, 20 to 40% EtOAc/Hex, another column 30 to 40% EtOAc/Hex) to give a

white solid (3.20 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (dd, J = 13.8, 8.2 Hz, 2 H), 7.41-7.10 (m, 14 H), 5.17 (br s, 1 H, N*H*), 4.35 (d, J = 5.2 Hz, 2 H), 1.46 (s, 9 H); ^{31}P NMR (121 MHz, $CDCl_3$): δ 11.8.

Example 25

Compound 32: To a solution of compound **31** (3.73 g, 8.49 mmol) in acetonitrile (85 mL) at 0°C was added 1 M NaOH (21.2 mL, 21.21 mmol). Reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature over 4 h. Reaction mixture cooled to 0°C and Dowex (H+) residue was added to pH 2. Mixture was filtered, concentrated and residue obtained was triturated with EtOAc/Hex (1:2) to give a white powder (2.889 g, 94%). This compound (2.00 g, 5.50 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL). The residue was dissolved in anhydrous pyridine (30 mL) and ethyl (S)-lactate (6.24 mL, 55 mmol) and reaction mixture was heated to 70°C. After 5 min, 1,3-dicyclocarbodiiimide (4.54 g, 22.0 mmol) was added. Reaction mixture was stirred at 70°C for 5 h, then cooled and concentrated. Residue was dissolved in EtOAc and precipitate was removed by filtration. The filtrate was concentrated and purified (25 to 35% EtOAc/Hex, another column 40% EtOAc/Hex) to give a colorless oil (2.02 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96-7.85 (m, 2 H), 7.42-7.35 (m, 2 H), 7.35-7.08 (m, 4 H), 5.16-5.00 (m, 1 H), 4.93 (s, 1 H, N*H*), 4.37 (d, J = 5.5 Hz, 1 H), 4.21 (q, J = 7.3 Hz, 1 H), 4.11 (dq, J = 5.7, 2.2 Hz, 1 H), 1.62-1.47 (m, 3 H), 1.47 (s, 9 H), 1.27 (t, J = 7.3 Hz, 1.5 H), 1.17 (t, J = 7.3 Hz, 1.5 H); ^{31}P NMR (121 MHz, $CDCl_3$): δ 16.1, 15.0.

Example 26

Compound 33: Compound **32** (2.02 g, 4.36 mmol) was dissolved in CH_2Cl_2 (41 mL) and cooled to 0°C. To this solution was added trifluoroacetic acid (3.5 mL) and reaction mixture was stirred at 0°C for 1 h, then at room temperature for 3 h. Reaction mixture was concentrated, coevaporated with EtOAc and diluted with H_2O (400 mL). Mixture was neutralized with Amberlite IRA-67 weakly basic resin, then filtered and concentrated. Coevaporation with MeOH and dried under high vacuum to give the TFA amine salt as a semi-solid (1.48 g, 94%). To a solution of the amine (1.48 g, 4.07 mmol) in absolute ethanol (20 mL) at 0°C was added aldehyde **24** (1.39 g, 2.26 mmol), followed by acetic acid (0.14 mL, 2.49 mmol). After stirring for 5 min, sodium cyanoborohydride (0.284 g, 4.52 mmol)

was added and reaction mixture stirred for 30 min at 0°C. Reaction was quenched with saturated $NaHCO_3$ solution and diluted with EtOAc and H_2O. Aqueous layer was extracted with EtOAc (3x) and combined organic layer was dried ($MgSO_4$), concentrated and purified (silica gel, 2 to 4% MeOH/CH_2Cl_2) to give white foam (0.727 g, 33%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98-7.86 (m, 2 H), 7.71 (d, J = 8.6 Hz, 2 H), 7.49 (br s, 2 H), 7.38-7.05 (m, 5 H), 6.98 (d, J = 8.8 Hz, 2 H), 5.72 (d, J = 5.1 Hz, 1 H), 5.28-5.00 (m, 2 H), 4.30-3.72 (m, 12 H), 3.42-3.58 (m, 1 H), 3.20-2.68 (m, 7 H), 2.25-1.42 (m, 6 H), 1.26 (t, J = 7.2 Hz, 1.5 H), 1.17 (t, J = 7.2 Hz, 1.5 H), 1.08-0.50 (m, 21 H); ^{31}P NMR (121 MHz, $CDCl_3$): δ 16.1, 15.1.

Example 27

Compound 34: To a solution of compound **33** (0.727 g, 0.756 mmol) in acetonitrile (7.6 mL) at 0°C was added 48% hydrofluoric acid (0.152 mL) and reaction mixture was stirred for 40 min at 0°C, then diluted with EtOAc and H_2O. Saturated $NaHCO_3$ was added and aqueous layer was extracted with EtOAc (2x). Combined organic layer was dried ($MgSO_4$), concentrated and purified (silica gel, 4 to 5% MeOH/CH_2Cl_2) to give a colorless foam (0.5655 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95-7.82 (m, 2 H), 7.67 (d, J = 8.1 Hz, 2 H), 7.41 (br s, 2 H), 7.38-7.05 (m, 5 H), 6.95 (d, J = 7.2 Hz, 2 H), 5.76 (d, J = 7.9 Hz, 1 H), 5.67 (d, J = 5.0 Hz, 1 H), 5.32-4.98 (m, 2 H), 4.25-3.75 (m, 13 H), 3.25-2.70 (m, 7 H), 2.15-1.76 (m, 3 H), 1.53-1.41 (m, 3 H), 1.25-1.08 (m, 3 H), 0.87 (d, J = 4.2 Hz, 6 H); ^{31}P NMR (121 MHz, $CDCl_3$): δ 16.1, 15.0.

Example 28

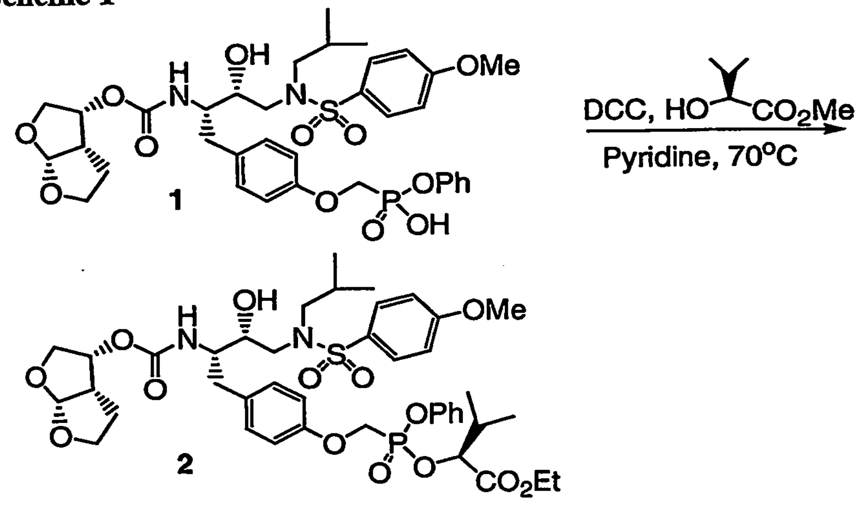
Compound 35: To a solution of compound **33** (0.560 g, 0.660 mmol) in absolute ethanol (13 mL) at 0°C was added 37% formaldehyde (0.54 mL, 6.60 mmol), followed by acetic acid (0.378 mL, 6.60 mmol). The reaction mixture was stirred at 0°C for 5 min, then sodium cyanoborohydride (0.415 g, 6.60 mmol) was added. Reaction mixture was warmed to room temperature over 2 h, then quenched with saturated $NaHCO_3$ solution. EtOAc was added and mixture was washed with brine. Aqueous layer was extracted with EtOAc (2x) and combined organic layer was dried ($MgSO_4$), concentrated and purified (silica gel, 3% MeOH/CH_2Cl_2) to give a white foam (0.384 g, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95-7.82 (m, 2 H), 7.71 (d, J = 8.4 Hz, 2 H), 7.38 (br s, 2 H), 7.34-7.10 (m, 5 H), 6.98 (d, J = 8.8 Hz, 2 H), 5.72 (d, J = 5.0 Hz, 1 H), 5.50 (br s, 1 H), 5.19-5.01 (m, 2 H), 4.29-3.75 (m, 10 H),

3.85 (s, 3 H), 3.35-2.70 (m, 7 H), 2.23 (s, 3 H), 2.17-1.79 (m, 3 H), 1.54 (d, $J = 6.9$ Hz, 1.5 H), 1.48 (d, $J = 6.8$ Hz, 1.5 H), 1.25 (t, $J = 7.2$ Hz, 1.5 H), 1.16 (t, $J = 7.2$ Hz, 1.5 H), 0.92 (d, $J = 6.6$ Hz, 3 H), 0.87 (d, $J = 6.6$ Hz, 3 H). ^{31}P NMR (121 MHz, $CDCl_3$): δ 16.0, 14.8.

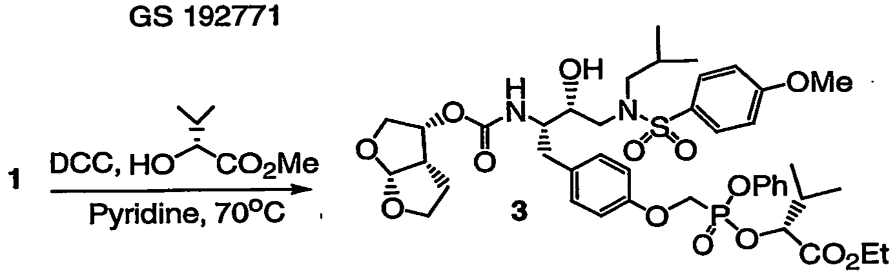
Example 29

Compound 36: To a solution of compound **35** (44 mg, 0.045 mmol) in acetonitrile (1.0 mL) and DMSO (0.5 mL) was added phosphate buffered saline (pH 7.4, 5.0 mL) to give a cloudy white suspension. Porcine liver esterase (200 μL) was added and reaction mixture was stirred for 48 h at 38°C. Additional esterase (600 μL) was added and reaction was continued for 4 d. Reaction mixture was concentrated, diluted with MeOH and the resulting precipitate removed by filtration. Filtrate was concentrated and purified by reverse phase HPLC to give a white powder after lyophilization (7.2 mg, 21%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.95 (br s, 2 H), 7.76 (d, $J = 8.4$ Hz, 2 H), 7.64 (br s, 2 H), 7.13 (d, $J = 8.7$ Hz, 2 H), 5.68 (d, $J = 5.1$ Hz, 1 H), 5.14 (br s, 1 H), 4.77 (br s, 1 H), 4.35-3.59 (m, 8 H), 3.89 (s, 3 H), 3.45-2.62 (m, 10 H), 2.36-1.86 (m, 3 H), 1.44 (d, $J = 6.3$ Hz, 3 H), 0.92 (d, $J = 6.6$ Hz, 3 H), 0.84 (d, $J = 6.6$ Hz, 3 H); ^{31}P NMR (121 MHz, CD_3OD): δ 13.8.

Scheme 1

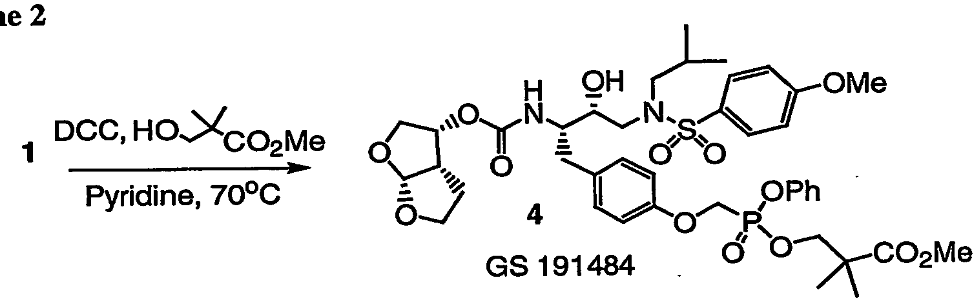


GS 192771

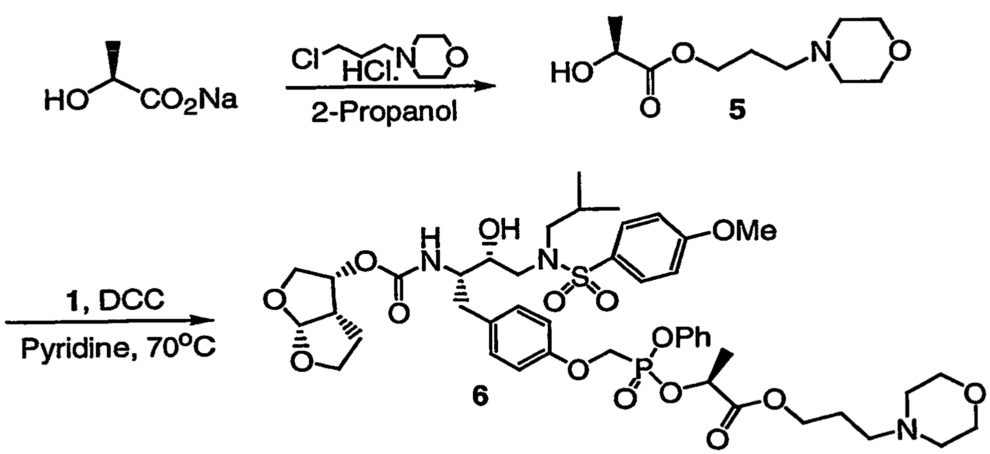


GS 192772

Scheme 2

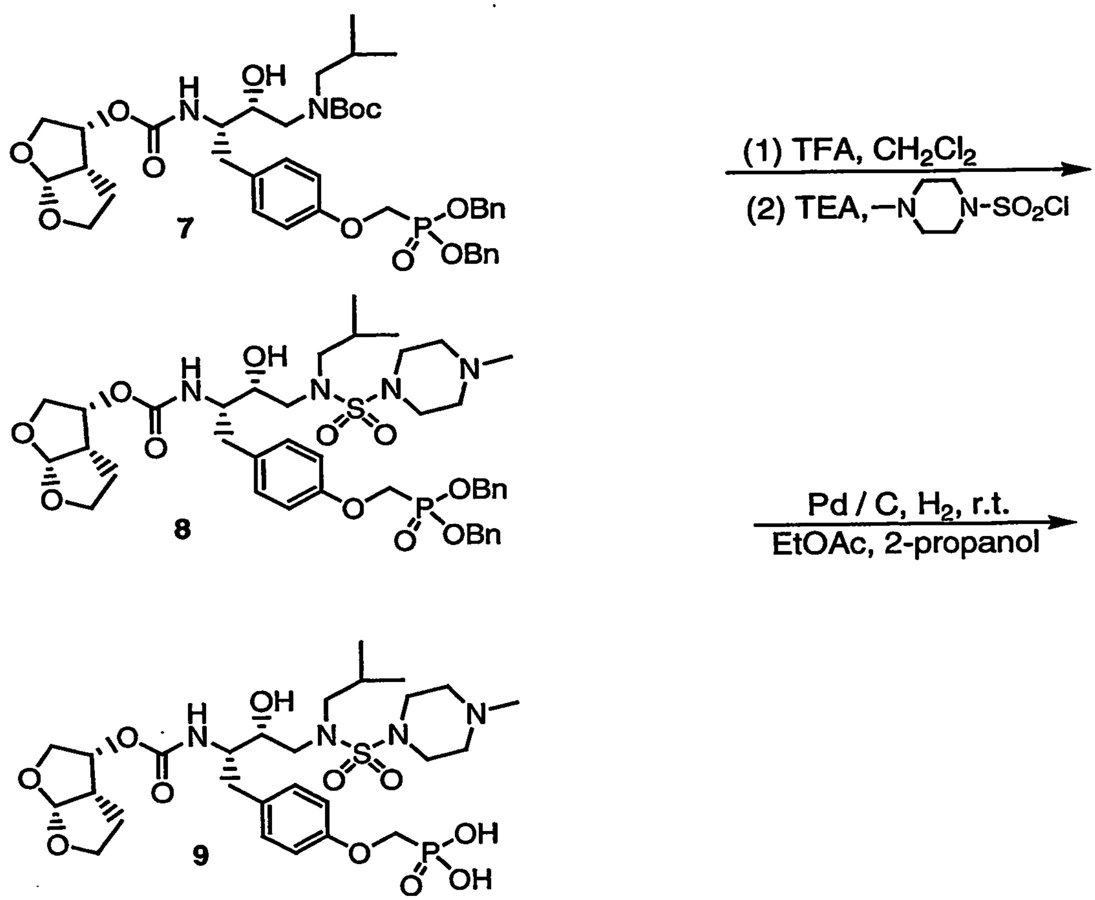


GS 191484

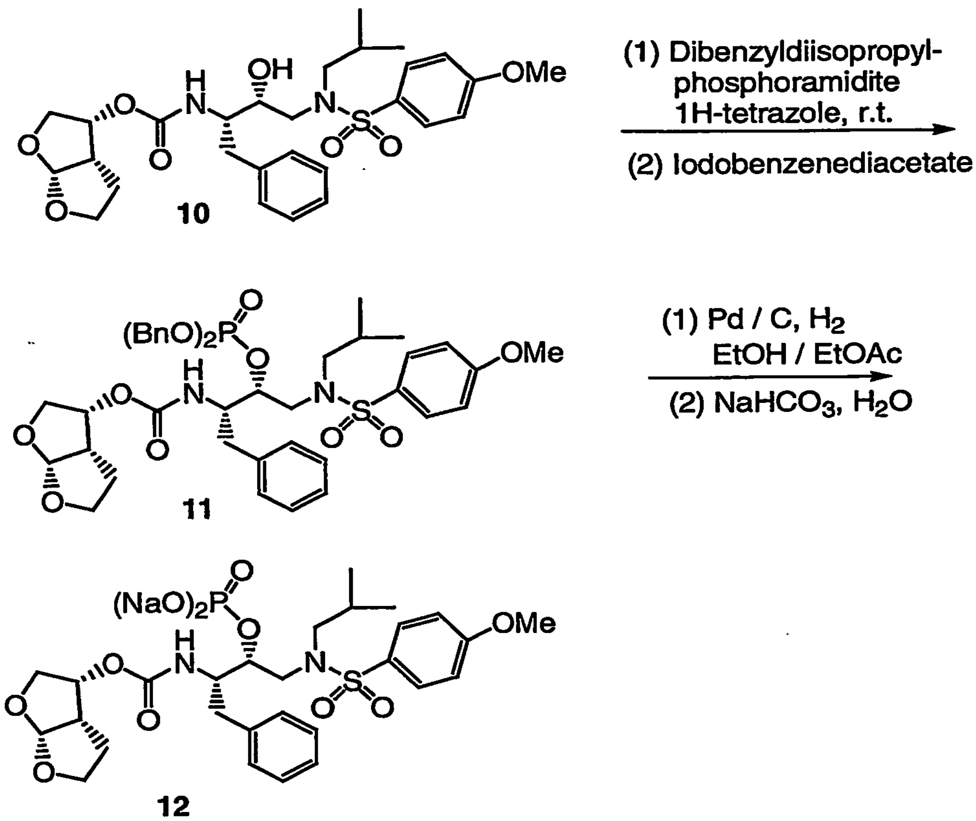


GS 192781

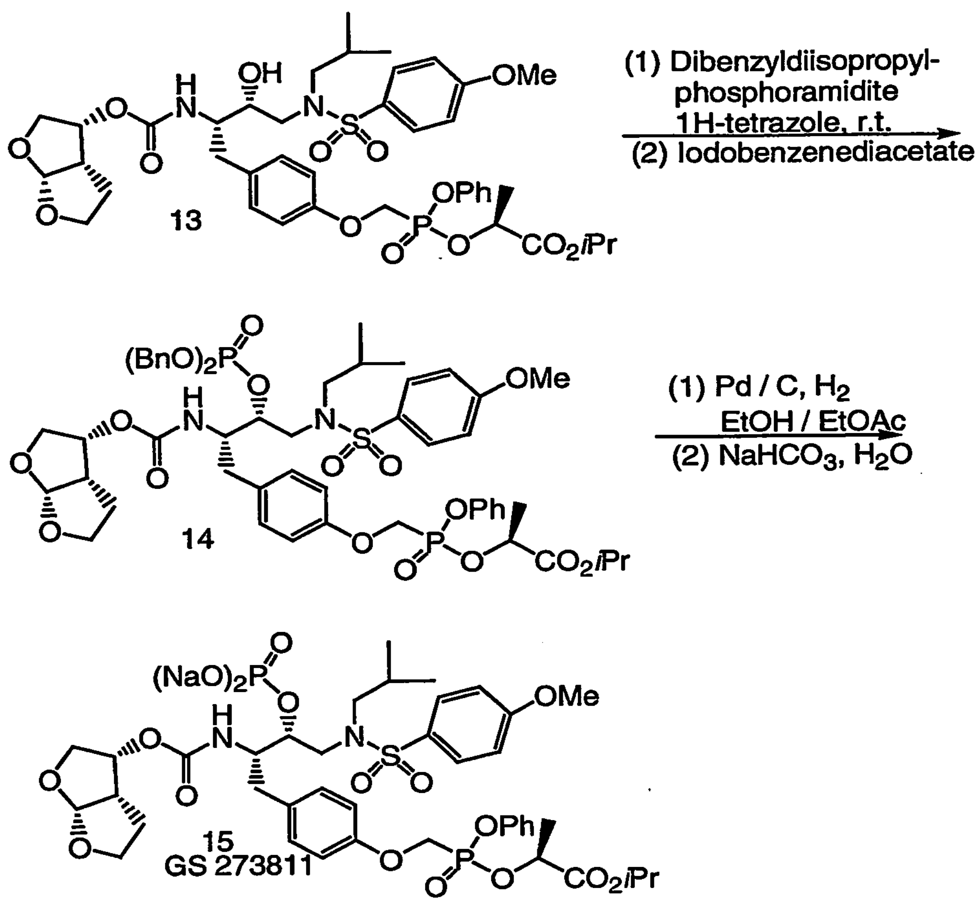
Scheme 3



Scheme 4


OMe
OH
H
N
O
S
10
(1) Dibenzyldiisopropyl-
phosphoramidite
1H-tetrazole, r.t.
(2) Iodobenzenediacetate
(BnO)2P
11
(1) Pd / C, H2
EtOH / EtOAc
(2) NaHCO3, H2O
(NaO)2P
12

Scheme 5



Example 1

Monophospholactate 2: A solution of 1 (0.11 g, 0.15 mmol) and α-hydroxyisovaleric acid ethyl-(S)-ester (71 mg, 0.49 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H_2O, saturated NaCl, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH_2Cl_2) to give the monophospholactate (35 mg, 28%, GS 192771, 1/1 diastereomeric mixture) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.7 Hz, 2H), 7.36-7.14 (m, 7H), 6.99 (d, J = 8.7 Hz, 2H), 6.94-6.84 (dd, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.00-4.85 (m, 3H), 4.55 (dd, 1H), 4.41 (dd, 1H), 4.22-4.07 (m, 2H), 3.96-3.68 (m, 9H), 3.12-2.74 (m, 7H), 2.29 (m, 1H), 1.85-1.57

(m, 3H), 1.24 (m, 3H), 1.05 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H), 0.9 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 17.7, 15.1.

Example 2

Monophospholactate 3: A solution of 1 (0.11 g, 0.15 mmol) and α-hydroxyisovaleric acid ethyl-(R)-ester (71 mg, 0.49 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H_2O, saturated NaCl, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH_2Cl_2) to give the monophospholactate (35 mg, 28%, GS 192772, 1/1 diastereomeric mixture) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.7 Hz, 2H), 7.35-7.13 (m, 7H), 6.98 (d, J = 8.7 Hz, 2H), 6.93-6.83 (dd, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.04-4.85 (m, 3H), 4.54 (dd, 1H), 4.39 (dd, 1H), 4.21-4.06 (m, 2H), 3.97-3.67 (m, 9H), 3.12-2.75 (m, 7H), 2.27 (m, 1H), 1.83-1.57 (m, 3H), 1.26 (m, 3H), 1.05 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H), 0.9 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 17.7, 15.1.

Example 3

Monophospholactate 4: A solution of 1 (0.10 g, 0.13 mmol) and methyl-2,2-dimethyl-3-hydroxypropionate (56 μL, 0.44 mmol) in pyridine (1 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (91 mg, 0.44 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H_2O, saturated NaCl, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH_2Cl_2) to give the monophospholactate (72 mg, 62%, GS 191484) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.7 Hz, 2H), 7.34 (m, 2H), 7.25-7.14 (m, 5H), 7.00 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.05 (m, 2H), 4.38 (d, J = 9.6 Hz, 2H),

4.32-4.20 (m, 2H), 4.00 (m, 2H), 3.87-3.63 (m, 12H), 3.12-2.78 (m, 7H), 1.85-1.67 (m, 3H), 1.20 (m, 6H), 0.91 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); ^{31}P NMR ($CDCl_3$) δ 16.0.

Example 4

Lactate 5: To a suspension of lactic acid sodium salt (5 g, 44.6 mmol) in 2-propanol (60 mL) was added 4-(3-chloropropyl)morpholine hydrochloride (8.30 g, 44.6 mmol). The reaction mixture was heated to reflux for 18 h and cooled to room temperature. The solid was filtered and the filtrate was recrystallized from EtOAc / hexane to give the lactate (1.2 g, 12%).

Example 5

Monophospholactate 6: A solution of 1 (0.10 g, 0.13 mmol) and lactate 5 (0.10 g, 0.48 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and H_2O. The EtOAc layer was washed with saturated NaCl, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/CH_2Cl_2) to give the monophospholactate (30 mg, 24%, GS 192781, 1/1 diastereomeric mixture) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.7 Hz, 2H), 7.38-7.15 (m, 7H), 7.00 (d, J = 8.7 Hz, 2H), 6.91 (m, 2H), 5.65 (d, J = 3.3 Hz, 1H), 5.18-4.98 (m, 3H), 4.54 (dd, 1H), 4.42 (dd, 1H), 4.2 (m, 2H), 4.00-3.67 (m, 16H), 3.13-2.77 (m, 7H), 2.4 (m, 5H), 1.85-1.5 (m, 5H), 1.25 (m, 2H), 0.93 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); ^{31}P NMR ($CDCl_3$) δ 17.4, 15.4.

Example 6

Sulfonamide 8: A solution of dibenzylphosphonate 7 (0.1 g, 0.13 mmol) in CH_2Cl_2 (0.5 mL) at 0°C was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH_2Cl_2 (1 mL) and cooled to 0°C. Triethylamine (72 μL, 0.52 mmol) was added followed by the treatment of 4-methylpiperazinylsulfonyl chloride (25 mg, 0.13 mmol). The solution was stirred for 1 h at

0°C and the product was partitioned between CH_2Cl_2 and H_2O. The organic phase was washed with saturated NaCl, dried with Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (5% 2-propanol/CH_2Cl_2) to give the sulfonamide 8 (32 mg, 30%, GS 273835) as a white solid: ^{1}HNMR ($CDCl_3$) δ 7.35 (m, 10H), 7.11 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.2-4.91 (m, 4H), 4.2 (d, J = 10.2 Hz, 2H), 4.0-3.69 (m, 6H), 3.4-3.19 (m, 5H), 3.07-2.75 (m, 5H), 2.45 (m, 4H), 2.3 (s, 3H), 1.89-1.44 (m, 7H), 0.93 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 20.3.

<u>Example 7</u>

Phosphonic Acid 9: To a solution of 8 (20 mg, 0.02 mmol) in EtOAc (2 mL) and 2-propanol (0.2 mL) was added 10% Pd/C (5 mg). The suspension was stirred under H_2 atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (10 mg, 64%) as a white solid.

<u>Example 8</u>

Dibenzylphosphonate 11: A solution of 10 (85 mg, 0.15 mmol) and 1*H*-tetrazole (14 mg, 0.20 mmol) in CH_2Cl_2 (2 mL) was treated with Dibenzyldiisopropylphosphoramidite (60 μL, 0.20 mmol) and stirred at room temperature overnight. The product was partitioned between CH_2Cl_2 and H_2O, dried with Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give the intermediate dibenzylphosphite (85 mg, 0.11 mmol) which was dissolved in CH_3CN (2 mL) and treated with iodobenzenediacetate (51 mg, 0.16 mmol). The reaction mixture was stirred at room temperature for 3 h and concentrated. The residue was partitioned between EtOAc and $NaHCO_3$. The organic layer was washed with H_2O, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH_2Cl_2) to give the dibenzylphosphonate (45 mg, 52%) as a white solid.

<u>Example 9</u>

Disodium Salt of Phosphonic Acid 12: To a solution of 11 (25 mg, 0.03 mmol) in EtOAc (2 mL) was added 10% Pd/C (10 mg). The suspension was stirred under H_2 atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of

celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid which was dissolved in H_2O (1mL) and treated with $NaHCO_3$ (2.53 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (19.77 mg, 95%, GS 273777) as a white solid: ^1H NMR (CD_3OD) δ 7.81 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 7.27-7.09 (m, 5H), 5.57 (d, J = 5.1 Hz, 1H), 5.07 (m, 1H), 4.87-4.40 (m, 3H), 3.93-3.62 (m, 6H), 3.45-2.6 (m, 6H), 2.0 (m, 2H), 1.55 (m, 1H), 0.95-0.84 (m, 6H).

Example 10

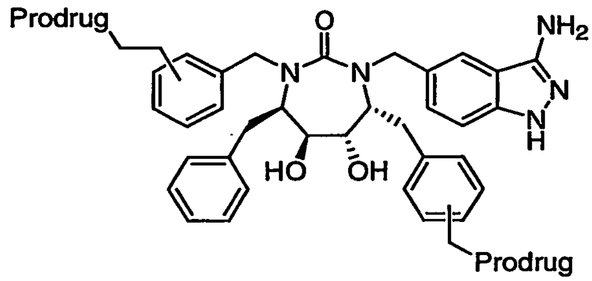
Dibenzylphosphonate 14: A solution of 13 (0.80 g, 0.93 mmol) and 1*H*-tetrazole (98 mg, 1.39 mmol) in CH_2Cl_2 (15 mL) was treated with dibenzyldiisopropylphosphoramidite (0.43 mL, 1.39 mmol) and stirred at room temperature overnight. The product was partitioned between CH_2Cl_2 and H_2O, dried with Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give the intermediate dibenzylphosphite (0.68 g, 67%). To a solution of the dibenzylphosphite (0.39 g, 0.35 mmol) in CH_3CN (5 mL) was added iodobenzenediacetate (0.17 g, 0.53 mmol). The reaction mixture was stirred at room temperature for 2 h and concentrated. The residue was partitioned between EtOAc and $NaHCO_3$. The organic layer was washed with H_2O, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH_2Cl_2) to give the dibenzylphosphonate (0.35 g, 88%) as a white solid.

Example 11

Disodium Salt of Phosphonic Acid 15: To a solution of 14 (0.39 g, 0.35 mmol) in EtOAc (30 mL) was added 10% Pd/C (0.10 g). The suspension was stirred under H_2 atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid, which was dissolved in H_2O (3 mL) and treated with $NaHCO_3$ (58 mg, 0.70 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (0.31 g, 90%, GS 273811) as a white solid: ^1H NMR (CD_3OD) δ 7.81 (d, J = 9.0 Hz, 2H), 7.43-7.2 (m, 7H), 7.13 (d, J = 9.0 Hz, 2H), 6.9 (m, 2H), 5.55 (d, J = 4.8 Hz, 1H), 5.07 (m, 2H), 4.87(m, 1H), 4.64-4.4 (m, 4H), 3.93-3.62 (m, 9H), 3.33-2.63 (m, 5H), 2.11 (m, 1H), 1.6-1.42 (m, 4H), 1.38-1.25 (m, 7H), 0.95 (d, J = 6.3 Hz, 3H), 0.84 (d, J = 6.3 Hz, 3H).

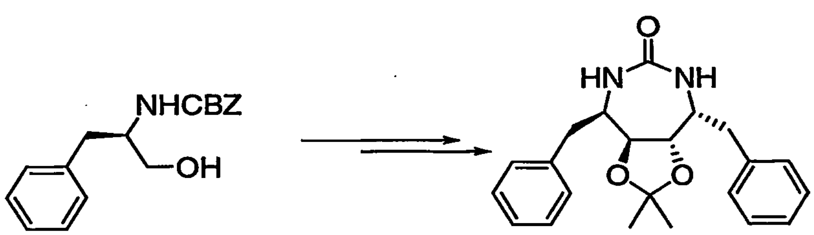
Examples For The Preparation Of Cyclic Carbonyl-Like Phosphonate Protease Inhibitors (CCPPI)

Phosphonamidate Prodrugs



Scheme 1-2	Scaffold Synthesis
Scheme 3-10	P2′-Benzyl ether phosphonates
Scheme 11-13	P2′-Alkyl ether phosphonates
Scheme 14-17	P2′-Benzyl Amide phosphonates
Scheme 18-25	P1-Phosphonates
Scheme 50	Reagents

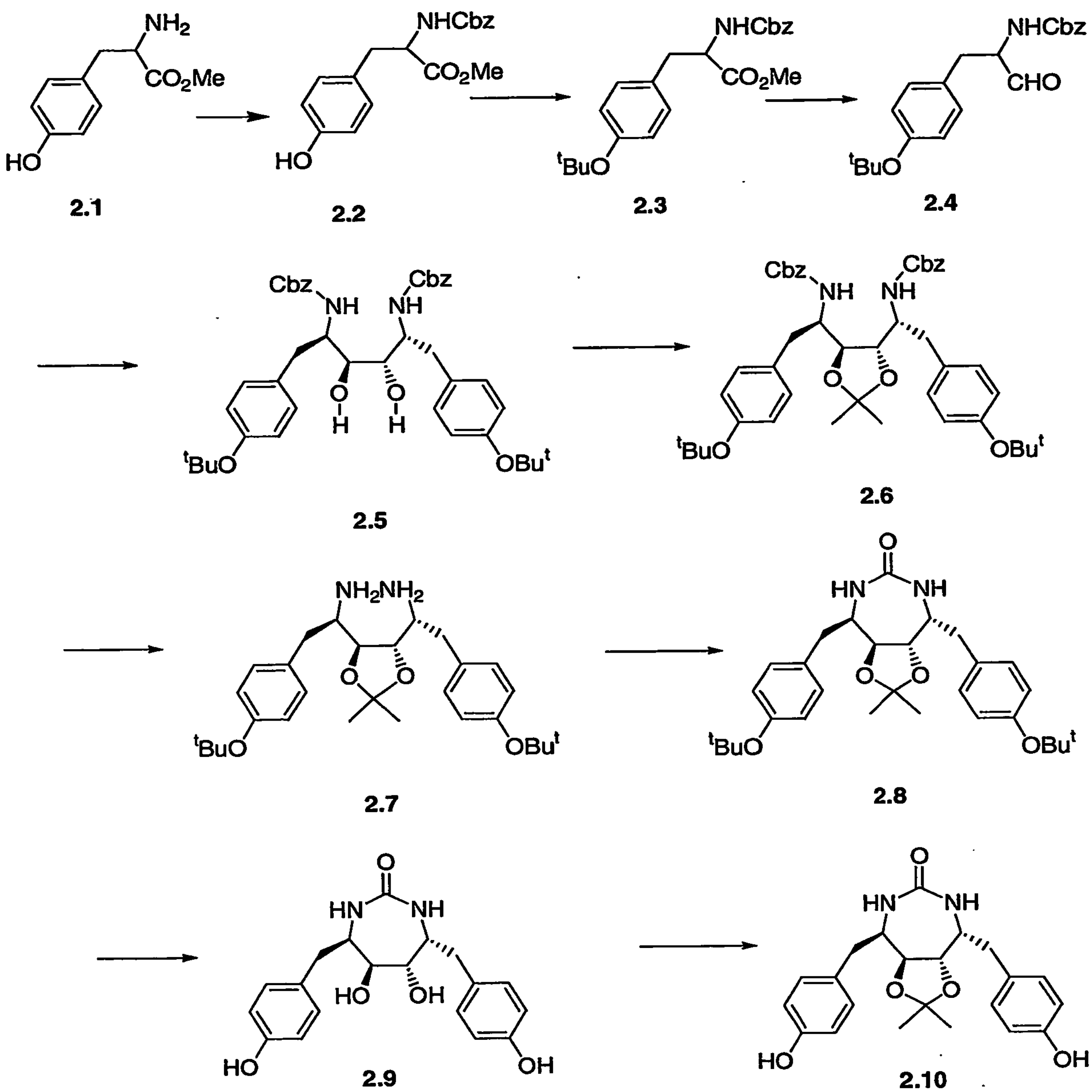
Scheme 1



1 1.1

The conversion of **1** to **1.1** is described in J. Org Chem 1996, 61, p444-450

Scheme 2



2-Benzyloxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionic acid methyl ester (2.3)
H-D-Tyr-O-me hydrochloride **2.1** (25 g, 107.7 mmol) is dissolved in methylene chloride (150 mL) and aqueous sodium bicarbonate (22 g in 150 mL water), and then cooled to 0°C. To this resulting solution benzyl chloroformate (20 g, 118 mmol) is slowly added. After complete addition, the resulting solution is warmed to room temperature ,and is then stirred for 2 h. The organic phase is separated, dried over Na_2SO_4, and concentrated under reduced pressure, to give the crude carbamate **2.2** (35g). The crude CBZ-Tyr-OMe product is

dissolved in methylene chloride (300 mL) containing concentrated H_2SO_4. Isobutene is bubbled though the solution for 6 h. The reaction is then cooled to 0°C, and neutralized with saturated $NaHCO_3$ aqueous solution. The organic phase is separated, dried, concentrated under reduced pressure, and purified by silica gel column chromatography to afford the tert-butyl ether **2.3** (25.7 g, 62 %).

[2-(4-tert-Butoxy-phenyl)-1-formyl-ethyl]-carbamic acid benzyl ester (2.4) (Reference J. O. C. 1997, 62, 3884).

To a stirred –78°C methylene chloride solution (60 mL) of **2.3**, DIBAL (82 mL of 1.5 M in toluene, 123 mmol) was added over 15 min. The resultant solution was stirred at –78°C for 30 min. Subsequently, a solution of EtOH/36 % HCl (9/1; 15 mL) is added slowly. The solution is added to a vigorously stirred aqueous HCl solution (600 mL, 1N) at 0°C. The layers are then separated, and the aqueous phase is extracted with cold methylene chloride. The combined organic phases are washed with cold 1N HCl aqueous solution, water, dried over Na_2SO_4, and then concentrated under reduced pressure to give the crude aldehyde **2.4** (20 g, 91 %).

[4-Benzyloxycarbonylamino-1-(4-tert-butoxy-benzyl)-5-(4-tert-butoxy-phenyl)-2,3-dihydroxy-pentyl]-carbamic acid benzyl ester (2.5)

To a slurry of $VCl_3(THF)_3$ in methylene chloride (150 mL) at room temperature is added Zinc powder (2.9 g, 44 mmol), and the resulting solution is then stirred at room temperature for 1 hour. A solution of aldehyde **2.4** (20 g, 56 mmol) in methylene chloride (100 mL) is then added over 10 min. The resulting solution is then stirred at room temperature overnight, poured into an ice-cold H_2SO_4 aqueous solution (8 mL in 200 mL), and stirred at 0°C for 30 min. The methylene chloride solution is separated, washed with 1N HCl until the washing solution is light blue. The organic solution is then concentrated under reduced pressure (solids are formed during concentration), and diluted with hexane. The precipitate is collected and washed thoroughly with a hexane/methylene chloride mixture to give the diol product **2.5**. The filtrate is concentrated under reduced pressure and subjected to silica gel chomatography to afford a further 1.5 g of **2.5**. (Total = 13 g, 65 %).

[1-{5-[1-Benzyloxycarbonylamino-2-(4-tert-butoxy-phenyl)-ethyl]-2,2-dimethyl-[1,3]dioxolan-4-yl}-2-(4-tert-butoxy-phenyl)-ethyl]-carbamic acid benzyl ester (2.6)

Diol **2.5** (5 g, 7 mmol) is dissolved in acetone (120 mL), 2,2-dimethoxypropane (20 mL), and pyridinium p-toluenesulfonate (120 mg, 0.5 mmol). The resulting solution is refluxed for 30 min., and then concentrated under reduced pressure to almost dryness. The resulting mixture is partitioned between methylene chloride and saturated $NaHCO_3$ aqueous solution, dried, concentrated under reduced pressure, and purified by silica gel column chomatography to afford isopropylidene protected diol **2.6** (4.8 g, 92 %).

4,8-Bis-(4-tert-butoxy-benzyl)-2,2-dimethyl-hexahydro-1,3-dioxa-5,7-diaza-azulen-6-one(2.8)

The diol **2.6** is dissolved in EtOAc/EtOH (10 mL/2 mL) in the presence of 10 % Pd/C and hydrogenated at atmospheric pressure to afford the diamino compound **2.7**. To a solution of crude **2.7** in 1,1,2,2-tetrachloroethane is added 1,1-carboxydiimidazole (1.05 g, 6.5 mmol) at room temperature. The mixture is stirred for 10 min, and the resulting solution is then added dropwise to a refluxing 1,1',2,2'-tetrachloroethane solution (150 mL). After 30 min., the reaction mixture is cooled to room temperature, and washed with 5 % citric acid aqueous solution, dried over Na_2SO4, concentrated under reduced pressure, and purified by silica gel column chomatography to afford the cyclourea derivative **2.8** (1.92 g, 60 % over 2 steps).

5,6-Dihydroxy-4,7-bis-(4-hydroxy-benzyl)-[1,3]diazepan-2-one (2.9)

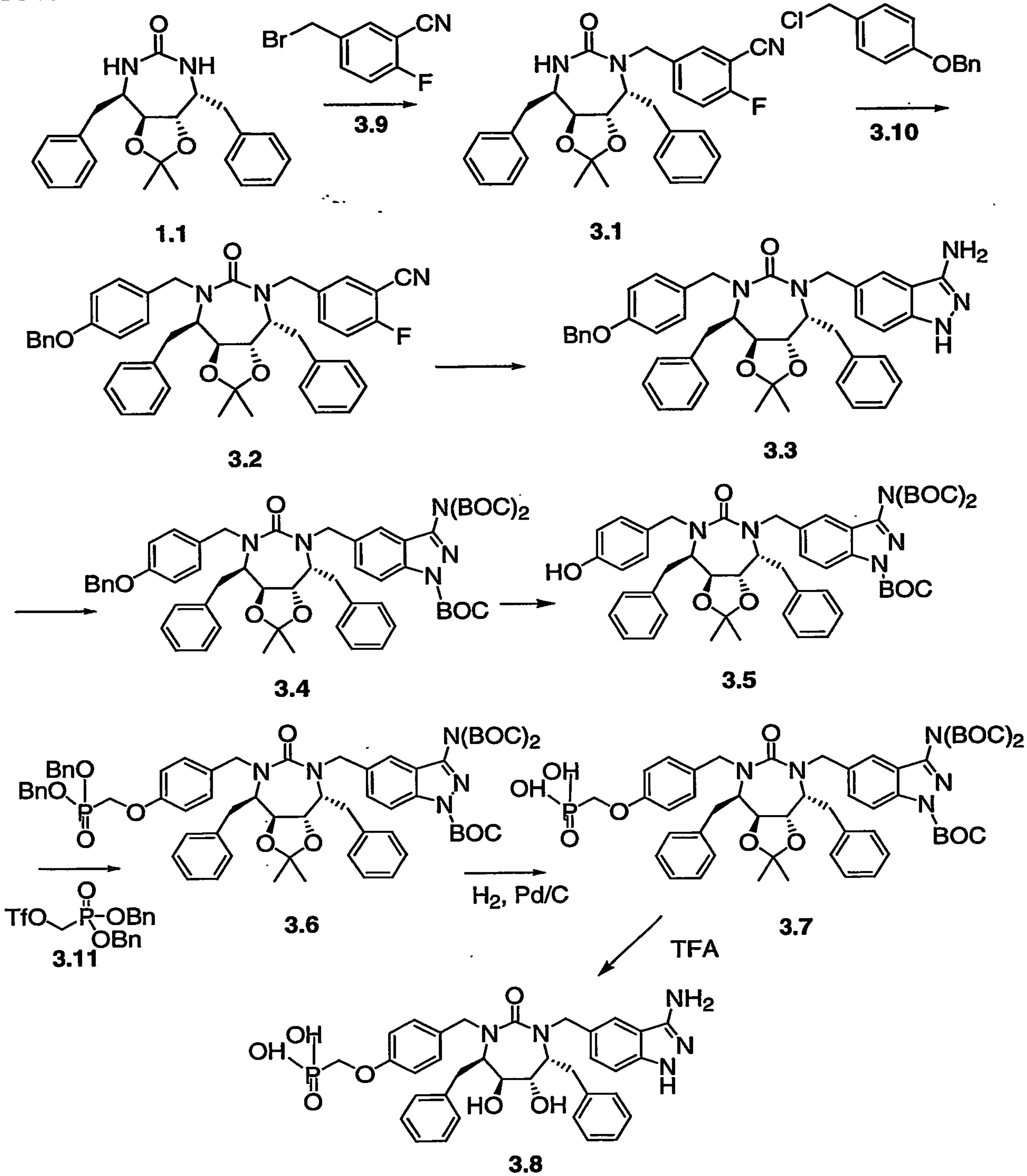
Cyclic Urea **2.8** (0.4 g, 0.78 mmol) was dissolved in dichloromethane (3 mL) and treated with TFA (1 mL). The mixture was stirred at room temperature for 2 h upon which time a white solid precipitated. 2 drops of water and methanol (2 mL) were added and the homogeneous solution was stirred for 1 h and concentrated under reduced pressure. The crude solid, **2.9**, was dried overnight and then used without further purification.

4,8-Bis-(4-hydroxy-benzyl)-2,2-dimethyl-hexahydro-1,3-dioxa-5,7-diaza-azulen-6-one (2.10)

Diol **2.9** (1.8 g, 5.03 mmol) was dissolved in DMF (6 mL) and 2,2-dimethoxypropane (12 mL). P-TsOH (95 mg) was added and the mixture stirred at 65°C for 3 h. A vacuum was applied to remove water and then the mixture was stirred at 65°C for a further 1 h. The excess dimethoxypropane was then distilled and the remaining DMF solution was then

allowed to cool. The solution of acetonide **2.10** can then used without further purification in future reactions.

Scheme 3



3-Cyano-4-fluorobenzyl urea 3.1 : A solution of urea **1.1** (1.6 g, 4.3 mmol) in THF was treated with sodium hydride (0.5 g of 60 % oil dispersion, 13 mmol). The mixture was stirred at room temperature for 30 min and then treated with 3-cyano-4-fluorobenzyl bromide **3.9** (1.0 g, 4.8 mmol). The resultant solution was stirred at room temperature for 3 h; concentrated under reduced pressure, and then partitioned between CH_2Cl_2 and saturated brine solution containing 1 % citric acid. The organic phase was separated , dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 15-25% ethyl acetate in hexanes to yield urea **3.1** (1.5 g, 69 %) as a white form.

Benzyl ether 3.2 : A solution of **3.1** (0.56 g, 1.1 mmol) in DMF (5 mL) was treated with sodium hydride (90 mg of 60 % oil dispersion, 2.2 mmol) and the resultant mixture stirred at room temperature for 30 min. 4-Benzyloxy benzyl chloride **3.10** (0.31 g, 1.3 mmol) was added and the resultant solution stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure and then partitioned between CH_2Cl_2 and saturated brine solution. The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel eluting with 1-10% ethyl acetate in hexanes to yield compound **3.2** (0.52 g, 67 %) as white form.

Indazole 3.3: Benzyl ether **3.2** (0.51 g, 0.73 mmol) was dissolved in n-butanol (10 mL) and treated with hydrazine hydrate (1 g, 20 mmol). The mixture was refluxed for 4 h and then allowed to cool to room temperature. The mixture was concentrated under reduced pressure and the residue was then partitioned between CH_2Cl_2 and 10 % citric acid solution. The organic phase was separated, concentrated under reduced pressure, and then purified by silica gel column eluting with 5% methanol in CH_2Cl_2 to afford indazole **3.3** (0.42 g, 82 %) as white solid.

Boc-indazole 3.4 : A solution of indazole **3.3** (0.4 g, 0.59 mmol) in CH_2Cl_2 (10 mL) was treated with diisopropylethylamine (0.19 g, 1.5 mmol), DMAP (0.18 g, 1.4 mmol), and di-tert-butyl dicarbonate (0.4 g, 2 mmol). The mixture was stirred at room temperature for 3 h and then partitioned between CH_2Cl_2 and 5 % citric acid solution. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The

residue was purified by silica gel eluting with 2% methanol in CH_2Cl_2 to afford **3.4** (0.42 g, 71 %).

Phenol 3.5 : A solution of **3.4** (300 mg, 0.3 mmol) in ethyl acetate (10 mL) and methanol (10 mL) was treated with 10 % Pd/C (40 mg) and stirred under a hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **3.5** as a white powder. This was used without further purification.

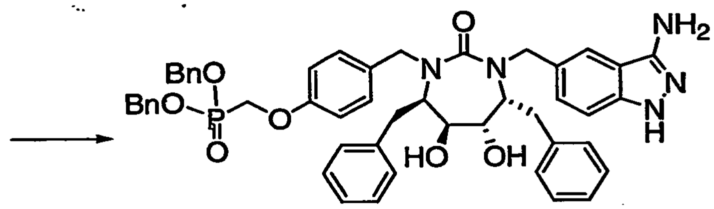
Dibenzyl ester 3.6 : A solution of **3.5** (0.1 mmol) in THF (5 mL) was treated with dibenzyl triflate **3.11** (90 mg, 0.2 mmol), and cesium carbonate (0.19 g, 0.3 mmol). The mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 20-40% ethyl acetate in hexanes to afford **3.6** (70 mg, 59 %). ^{1}H NMR ($CDCl_3$): δ 8,07 (d, 1H), 7.20-7.43 (m, 16H), 7.02-7.15 (m, 8 H), 6.80 (d, 2H), 5.07-5.18 (m, 4H), 5.03 (d, 1H), 4.90 (d, 1H), 4.20 (d, 2H), 3.74-3.78 (m, 4H), 3.20 (d, 1H), 3.05 (d, 1H) 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.40 (s, 18H), 1.26 (s, 6H); ^{31}P NMR ($CDCl_3$): 20.5 ppm.

Phosphonic acid 3.7: A solution of dibenzylphosphonate **3.6** (30 mg) in EtOAc (10 mL) was treated with 10% Pd/C (10 mg) and the mixture was stirred under a hydrogen atmosphere (balloon) for 3 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford phosphonic acid **3.7**. This was used without further purification.

Phosphonic acid 3.8: The crude phosphonic acid **3.7** was dissolved in CH_2Cl_2 (2 mL) and treated with trifluoroacetic acid (0.4 mL). The resultant mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and then purified by preparative HPLC (35 % CH_3CN/65 % H_2O) to afford the phosphonic acid **3.8** (9.4 mg, 55 %). ^{1}H NMR (CD_3OD): δ 7.71 (s, 1H), 7.60 (d, 1H), 6.95-7.40 (m, 15H), 4.65 (d, 2H), 4.17 (d, 2H), 3.50-3.70 (m, 3H), 3.42 (d, 1H), 2.03-3.14 (m, 6H); ^{31}P NMR ($CDCl_3$): 17.30

Scheme 4

3.6

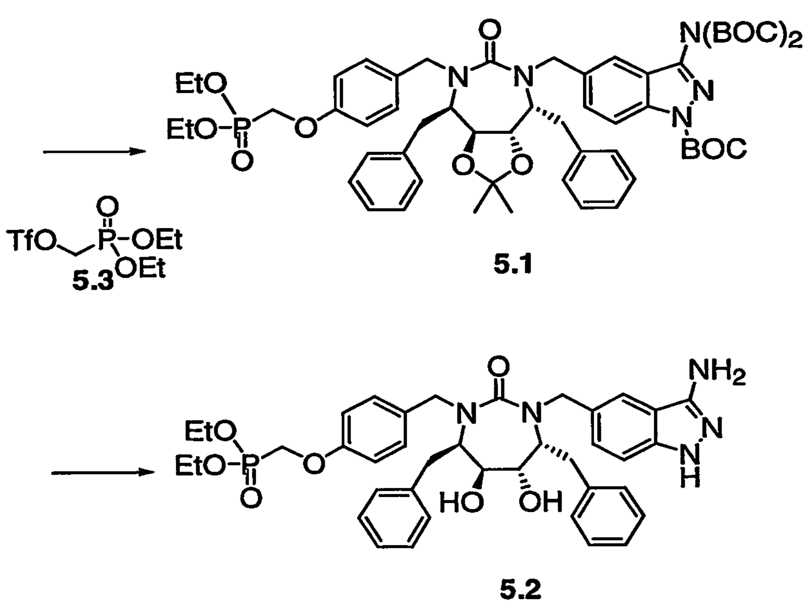


4.1

Dibenzylphosphonate 4.1: A solution of **3.6** (30 mg, 25 µmol) in CH_2Cl_2 (2 mL) was treated with TFA (0.4 mL) and the resultant mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel eluting with 50% ethyl acetate in hexanes to afford **4.1** (5 mg, 24%). ^{1}H NMR ($CDCl_3$): δ 6.96-7.32 (m, 25H), 6.95 (d, 2H), 5.07-5.18 (m, 4H), 4.86 (d, 1H), 4.7 5 (d, 1H), 4.18 (d, 2H), 3.40-3.62 (m, 4H), 3.25 (d, 1H), 2.80-3.15 (m, 6H); ^{31}P NMR ($CDCl_3$) 20.5 ppm; MS : 852 (M + H), 874 (M + Na).

Scheme 5

3.5



5.3

5.1

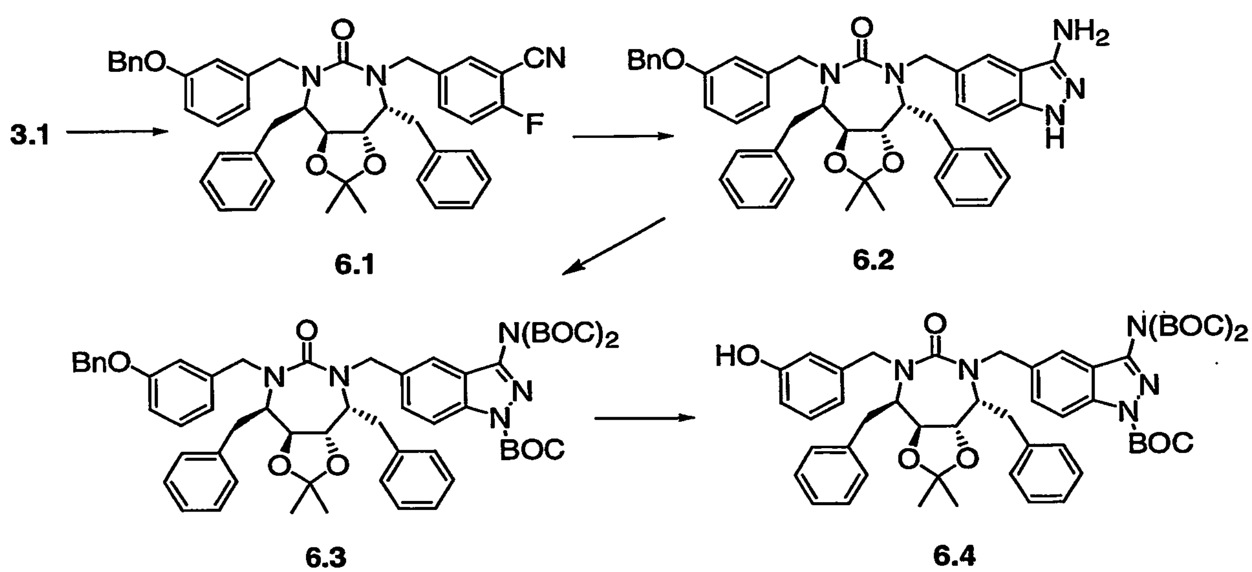
5.2

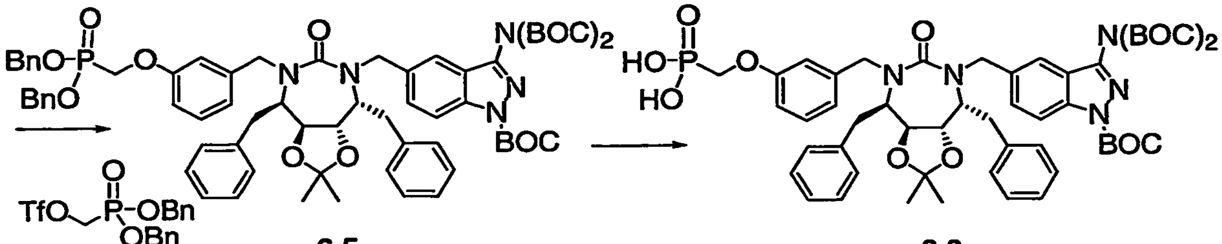
Diethylphosphonate 5.1: A solution of phenol **3.5** (48 mg, 52 µmol) in THF (5 mL) was treated with triflate **5.3** (50 mg, 165 µmol), and cesium carbonate (22 mg, 0.2 mmol). The

resultant mixture was stirred at room temperature for 5 h and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 7% methanol in CH_2Cl_2 to afford **5.1** (28 mg, 50 %). ^1H NMR ($CDCl_3$): δ 8,06 (d, 1H), 7.30-7.43 (m, 7H), 7.02-7.30 (m, 7 H), 6.88 (d, 2H), 5.03 (d, 1H), 4.90 (d, 1H), 4.10-4.25 (m, 6H), 3.64-3.80 (m, 4H), 3.20 (d, 1H), 3.05 (d, 1H) 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.20-1.50 (m, 30H); ^{31}P NMR ($CDCl_3$): 18.5 ppm; MS :1068 (M + H), 1090 (M + Na).

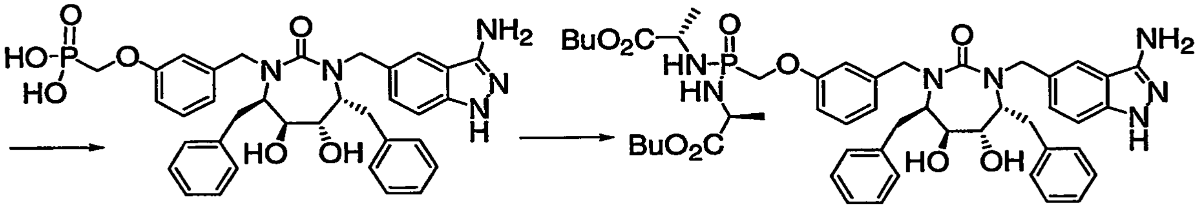
Diethylphosphonate 5.2: A solution of **5.1** (28 mg, 26 μmol) in CH_2Cl_2 (2 mL) was treated with TFA (0.4 mL) and the resultant mixture was stirred at room temperature for 4 hrs. The mixture was concentrated under reduced pressure and the residue was purified by silica gel to afford **5.2** (11 mg, 55 %). ^1H NMR ($CDCl_3$ + 10 % CD_3OD): δ 6.96-7.35 (m, 15H), 6.82 (d, 2H), 4.86(d, 1H), 4.75 (d, 1H), 4.10-4.23 (M, 6H), 3.40-3.62 (m, 4H), 2.80-3.20 (m), 1.31 (t, 6 H); ^{31}P NMR ($CDCl_3$ + 10 % CD_3OD): 19.80 ppm; MS : 728 (M + H).

Scheme 6





3.11 6.5 6.6



6.7 6.8.

3-Benzyloxybenzyl urea 6.1 : The urea **3.1** (0.87 g, 1.7 mmol) was dissolved in DMF and treated with sodium hydride (60% dispersion, 239 mg, 6.0 mmol) followed by m-benzyloxybenzylbromide **6.9** (0.60 g, 2.15 mmol). The mixture was stirred for 5 h and then diluted with ethyl acetate. The solution was washed with water, brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 25% ethyl acetate in hexanes to afford urea **6.1** (0.9 g, 75%).

Indazole 6.2: The urea **6.1** (41 mg, 59 μmol) was dissolved in n-butanol (1.5 mL) and treated with hydrazine hydrate (100 μL, 100 mmol). The mixture was refluxed for 2 h and then allowed to cool. The mixture was diluted with ethyl acetate, washed with 10% citric acid solution, brine, saturated $NaHCO_3$, and finally brine again. The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure to give the crude product **6.2** (35 mg, 83%). (Chem. Biol. 1998, 5, 597-608).

Boc-indazole 6.3 : The indazole **6.2** (1.04 g, 1.47 mmol) was dissolved in CH_2Cl_2 (20 mL) and treated with di-t-butyl dicarbonate (1.28 g, 5.9 mmol), DMAP (0.18 g, 1.9 mmol) and DIPEA (1.02 ml, 9.9 mmol). The mixture was stirred for 3 h and then diluted with ethyl acetate. The solution was washed with 5% citric acid solution, $NaHCO_3$, brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 50% ethyl acetate in hexanes to give **6.3** (0.71 g, 49%).

Phenol 6.4 : Compound **6.3** (20 mg, 0.021 mmol) was dissolved in MeOH (1 mL) and EtOAc (1 mL) and treated with 10% Pd/ C catalyst (5 mg). The mixture was stirred under a hydrogen atmosphere (balloon) until completion. The catalyst was removed by filtration and the filtrate concentrated under reduced pressure to afford compound **6.4** (19 mg, 100%).

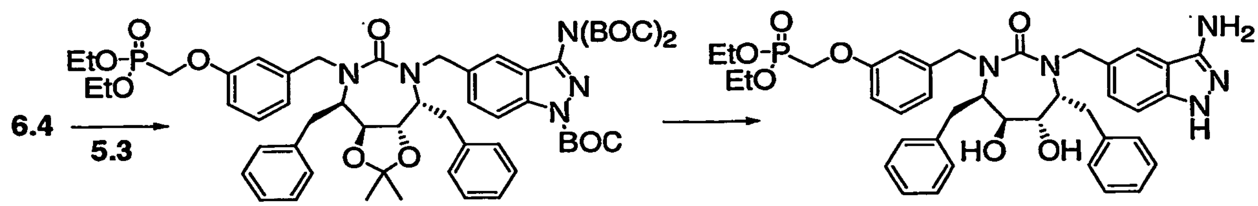
Dibenzyl phosphonate 6.5: A solution of compound **6.4** (0.34 g, 0.37 mmol) in acetonitrile (5 mL) was treated with Cs_2CO_3 (0.36 g, 1.1 mmol) and triflate **3.11** (0.18 mL, 0.52 mmol). The reaction mixture was stirred for 1 h. The reaction mixture was filtered and the filtrate was then concentrated under reduced pressure. The residue was re-dissolved in EtOAc, washed with water, saturated $NaHCO_3$, and finally brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with hexane: EtOAc (1:1) to afford compound **6.5** (0.32 g, 73%).

Phosphonic acid 6.6: Compound **6.5** (208 mg, 0.174 mmol) was treated in the same manner as benzyl phosphonate **3.6** in the preparation of phosphonate diacid **3.7**, except MeOH was used as the solvent, to afford compound **6.6** (166 mg, 94%).

Phosphonic acid 6.7: Compound **6.6** (89 mg, 0.088 mmol) was treated according to the conditions described in Scheme 3 for the conversion of **3.7** into **3.8**. The residue was purified by preparative HPLC eluting with a gradient of 90% methanol in 100 mM TEA bicarbonate buffer and 100% TEA bicarbonate buffer to afford phosphonic acid **6.7** (16 mg, 27%)

Bisamidate 6.8 : Triphenylphosphine (112 mg, 0.43 mmol) and aldrithiol-2 (95 mg, 0.43 mmol) were mixed in dry pyridine (0.5 mL). In an adjacent flask the diacid **6.7** (48 mg, 0.71 mmol) was suspended in dry pyridine (0.5 mL) and treated with DIPEA (0.075 mL 0.43 mmol) and L-AlaButyl ester hydrochloride (78 mg, 0.43 mmol) and finally the triphenylphosphine, aldrithiol-2 mixture. The reaction mixture was stirred under nitrogen for 24 h then concentrated under reduced pressure. The residue was purified by preparative HPLC eluting with a gradient of 5% to 95% acetonitrile in water. The product obtained was then further purified by silica gel eluting with CH_2Cl_2 : MeOH (9:1) to give compound **6.8** (9 mg, 14%).

Scheme 7

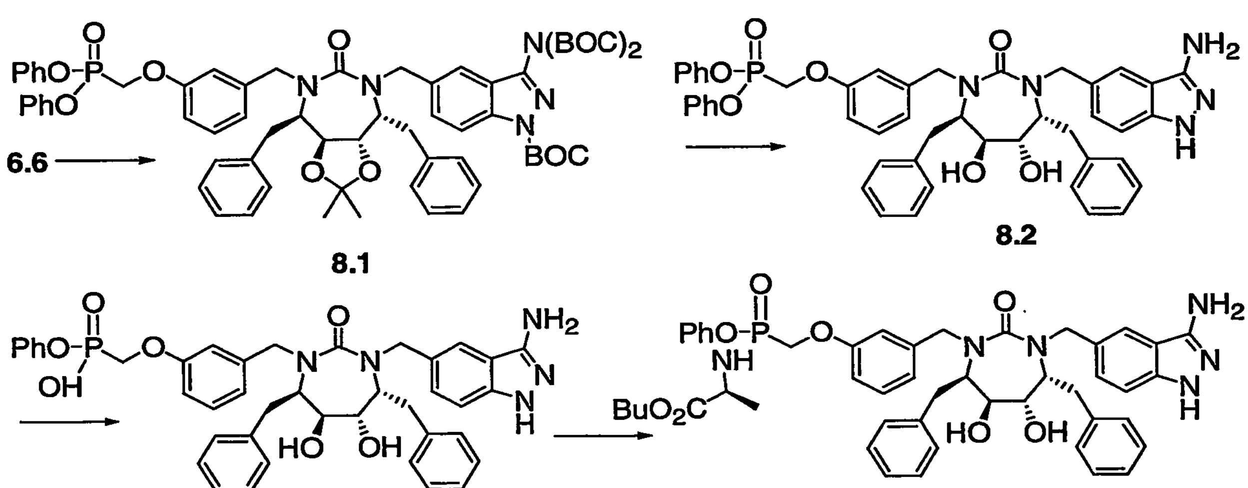


7.1 7.2

Diethyl phosphonate 7.1 : Compound **6.4** (164 mg, 0.179 mmol) was treated according to the procedure used to generate compound **6.5** except triflate **5.3** was used in place of triflate **3.11** to afford compound **7.1** (142 mg, 74%).

Diethylphosphonate 7.2 : Compound **7.1** (57 mg, 0.053 mmol) was treated according to the conditions used to form **6.7** from **6.6**. The residue formed was purified by silica gel eluting with CH_2Cl_2 : MeOH (9:1) to afford compound **7.2** (13 mg, 33%).

Scheme 8



8.1 8.2 8.3 8.4

Diphenylphosphonate 8.1: A solution of **6.6** (0.67g, 0.66 mmol) in pyridine (10 mL) was treated with phenol (0.62 g, 6.6 mmol) and DCC (0.82 mg, 3.9 mmol). The resultant mixture was stirred at room temperature for 5 min and then the solution was heated at 70°C for 3 h. The mixture was allowed to cool to room temperature and then diluted with EtOAc and water

(2 mL). The resultant mixture was stirred at room temperature for 30 min and then concentrated under reduced pressure. The residue was triturated with CH_2Cl_2, and the white solid that formed was removed by filtration. The filtrate was concentrated under reduced pressure and the resultant residue was purified by silica gel eluting with 30% ethyl acetate in hexanes to yield **8.1** (0.5 g, 65 %). ^{1}H NMR ($CDCl_3$): δ 8,08 (d, 1H), 7.41 (d, 1H), 7.05-7.35 (m, 22H), 6.85 (d, 2H), 6.70 (s, 1H). 5.19 (d, 1H), 5.10 (d, 1H), 4.70 (d, 2H), 3.70-3.90 (m, 4H), 3.20 (d, 1H), 3.11 (d, 1H), 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.40 (s, 18H), 1.30 (s, 6H); ^{31}P NMR ($CDCl_3$): 12.43 ppm

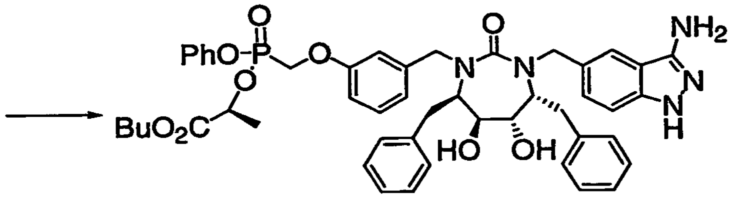
Diphenylphosphonate 8.2 : A solution of **8.1** (0.5 g, 0.42 mmol) in CH_2Cl_2 (4 mL) was treated with TFA (1 mL) and the resultant mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and azeotroped twice with CH_3CN. The residue was purified by silica gel eluting with 5% methanol in CH_2Cl_2 to afford diphenylphosphonate **8.2** (0.25 g, 71 %). ^{1}H NMR ($CDCl_3$): δ 7.03-7.40 (m, 21H), 6.81-6.90 (m, 3H), 4.96 (d, 1H), 4.90 (d, 1H) 4.60-4.70 (m, 2H), 3.43-3.57 (m, 4H), 3.20 (d, 1H), 2.80-2.97 (m, 5H); ^{31}P NMR ($CDCl_3$): 12.13 ppm; MS : 824 (M + H).

Monophenol 8.3 : The monophenol **8.3** (124 mg, 68 %) was prepared from the diphenol **8.2** by treating with 1N NaOH in acetonitrile at 0°C.

Monoamidate 8.4 : To a pyridine solution (0.5 mL) of **8.3** (40 mg, 53 μmol), n-butyl amidate HCl salt (116 mg, 640 μmol), and DIPEA (83 mg, 640 μmol) was added a pyridine solution (0.5 mL) of triphenyl phosphine (140 mg, 640 μmol), and aldrithiol-2 (120 mg, 640 μmol). The resulting solution was stirred at 65°C overnight, worked up, and purified by preparative TLC twice to give **8.4** (1.8 mg). δ 4.96 (d, 1H), 4.90 (d, 1H) 4.30-4.6 (m, 2H), 3.9-4.2 (m, 2H), 3.6-3.70 (m, 4H), 3.2-3.3 (d, 1H), 2.80-3.1 (m, 4H); MS: 875 (M + H) & 897 (M + Na)

Scheme 9

8.3

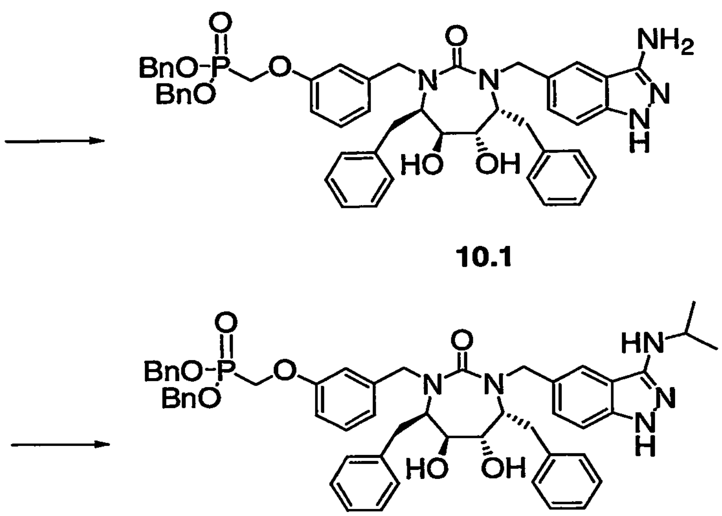


9.1

Monolactate **9.1**: The monolactate **9.1** is prepared from **8.3** using the conditions described above for the preparation of the monoamidate **8.4** except n-butyl lactate was used in place of n-butyl amidate HCl salt.

Scheme 10

6.5



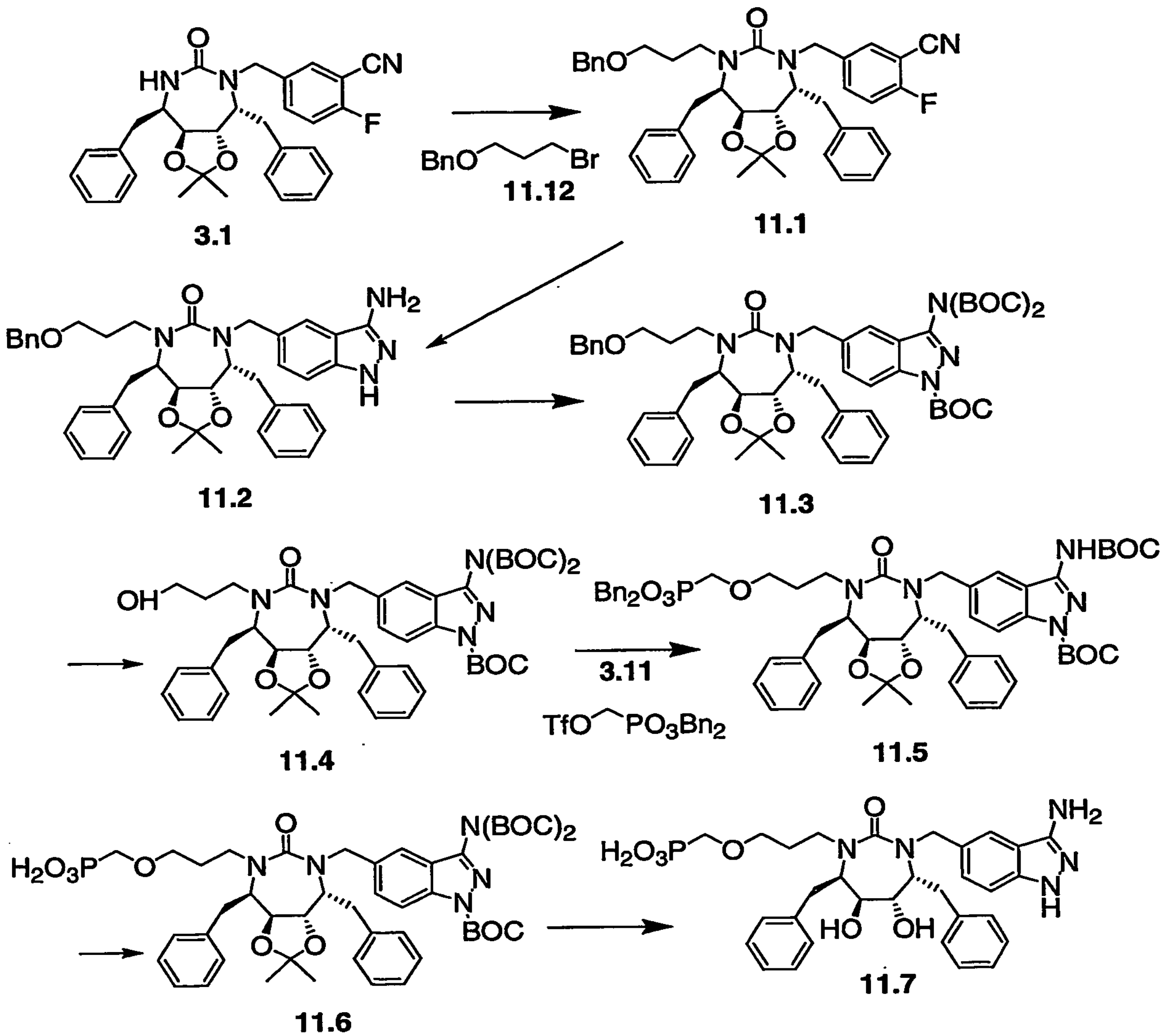
10.1

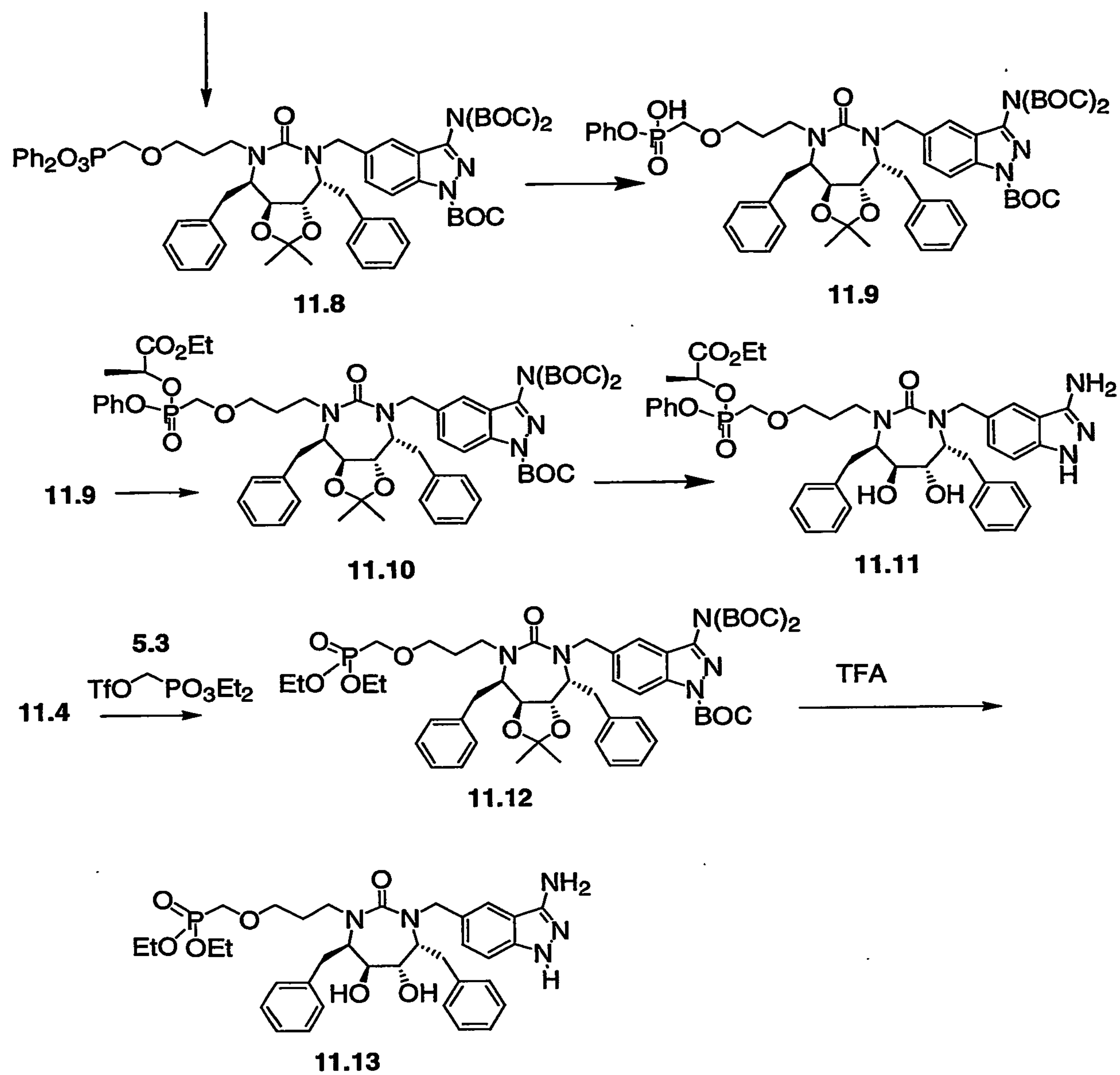
10.2

Dibenzylphosphonate 10.1: Compound **6.5** (16 mg, 0.014 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0°C. TFA (1 mL) was added and the reaction mixture was stirred for 0.5 h. The mixture was then allowed to warm to room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and azeotroped with toluene. The residue was purified by silica gel eluting with CH_2Cl_2 : MeOH (9:1) to afford compound **10.1** (4 mg, 32%).

Isopropylamino indazole 10.2 : Compound **10.1** (30 mg, 0.35 mmol) was treated with acetone according to the method of Henke et al. (J. Med Chem. 40 17 (1997) 2706-2725) to yield **10.2** as a crude residue. The residue was purified by silica gel eluting with CH_2Cl_2 : MeOH (93:7) to afford compound **10.2** (3.4 mg, 10%).

Scheme 11





Benzyl ether 11.1: A DMF solution (5 mL) of **3.1** (0.98 g, 1.96 mmol) was treated with NaH (0.24 g of 60 % oil dispersion, 6 mmol) for 30 min, followed by the addition of sodium iodide (0.3 g, 2 mmol), and benzoxypropyl bromide (0.55 g, 2.4 mmol). After the reaction for 3 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **11.1** (0.62 g, 49 %).

Aminoindazole 11.2: A n-butanol solution (10 mL) of **11.1** (0.6 g, 0.92 mmol) and hydrazine hydrate (0.93 g, 15.5 mmol) was heated at reflux for 4 h. The reaction mixture was concentrated under reduced pressure to give crude **11.2** (~0.6 g).

Tri-BOC-Aminoindazole 11.3: A methylene chloride solution (10 mL) of crude **11.2**, DIPEA (0.36 g, 2.8 mmol), $(BOC)_2O$ (0.73 g, 3.3 mmol), and DMAP (0.34 g, 2.8 mmol) was stirred for 5 h at room temperature, partitioned between methylene chloride and 5 % citric acid solution, dried, purified by silica gel column chomatography to give **11.3** (0.51 g, 58 %, 2 steps).

3-Hydroxypropyl cyclic urea 11.4: An ethyl acetate/ethanol solution (30 mL/5 mL) of **11.3** (0.5 g, 0.52 mmol) was hydrogenated at 1 atm in the presence of 10 % Pd/C (0.2 g) for 4 h. The catalyst was removed by filtration. The filtrate was then concentrated under reduced pressure to afford crude **11.4** (0.44 g, 98 %).

Dibenzyl phosphonate 11.5: A THF solution (3 mL) of **11.4** (0.5 g, 0.57 mmol) and triflate dibenzyl phosphonate **3.11** (0.37 g, 0.86 mmol) was cooled to –3°C, followed by addition of n-BuLi (0.7 mL of 2.5 M hexane solution, 1.7 mmol). After 2 h reaction, the reaction mixture was partitioned between methylene chloride and saturated NaCl solution, concentrated under reduced pressure. The residue was redissolved in methylene chloride (10 mL), and reacted with $(BOC)_2O$ (0.15 g, 0.7 mmol) in the presence of DMAP (0.18 g, 0.57 mmol), DIPEA (0.18 g, 1.38 mmol) for 2 h at room temperature. The reaction mixture was worked up, and purified by silica gel chromatography to give **11.5** (0.25 g, 43 %).

Phosphonic diacid 11.7: An ethyl acetate solution (2 mL) of **11.5A** (11 mg, 10.5 μmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (10 mg) for 6 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give crude **11.6**. The crude **11.6** was redissolved in methylene chloride (1 mL) and treated with TFA (0.2 mL) for 4 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by HPLC to give **11.7** (2 mg, 30%).
NMR (CD_3OD): δ 7.1-7.3 (m, 11H), 7.0-7.1 (d, 2H), 4.95 (d, 1H), 3.95-4.1 (d, 1H), 2.9 -3.3 (m, 4H), 2.3-2.45 (m, 1H), 1.6-1.8 (m, 2H). P NMR (CD_3OD):15.5 ppm. MS: 624 (M + 1).

Diphenyl phosphonate 11.8: A pyridine solution (1 mL) of **11.6** (0.23 g, 0.23 mmol), phenol (0.27 g, 2.8 mmol), and DCC (0.3 g, 1.4 mmol) was stirred for 5 min. at room temperature, then reacted at 70°C for 3 h. The reaction mixture was cooled to room

temperature, concentrated under reduced pressure, and purified by silica gel column chromatograph to afford **11.8** (0.11g, 41 %).

Monophenyl phosphonate 11.9: An acetonitrile solution (2 mL) of **11.8** (0.12 g, 0.107 mmol) at 0°C was treated with 1N sodium hydroxide aqueous solution (0.2 mL) for 1.5 h., then acidified with Dowex (50wx8-200, 120 mg). The Dowex was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was triturated with 10 % EtOAc/90 % hexane twice to afford **11.9** (90 mg, 76 %) as a white solid .

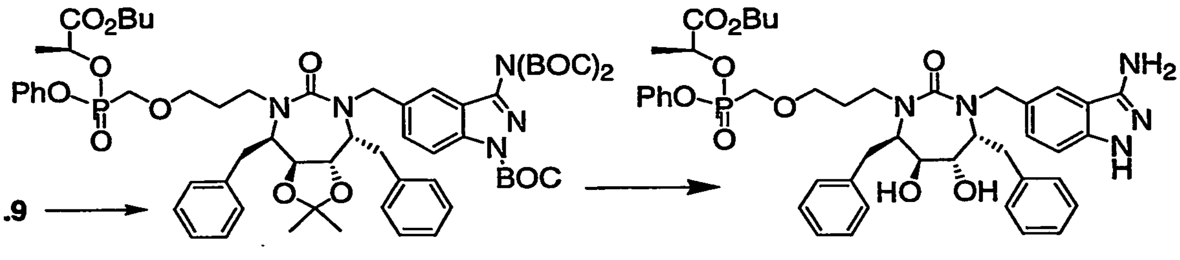
Mono-ethyl lactate phosphonate 11.10: A pyridine solution (0.3 mL) of **11.9** (33 mg, 30 μmol), ethyl lactate (41 mg, 340 μmol), and DCC (31 mg, 146 μmol) was stirred at room temperature for 5 min, then reacted at 70°C for 1.5 h. The reaction mixture was concentrated under reduced pressure, partitioned between methylene chloride and saturated NaCl solution, and purified by silica gel chromatography to give **11.10** (18 mg, 50 %).

Ethyl lactate phosphonate 11.11: A methylene chloride solution (0.8 mL) of **11.10** (18 mg, 15.8 μmol) was treated with TFA (0.2 mL) for 4 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give **11.11** (6 mg, 50 %). NMR ($CDCl_3$ + ~10 %CD_3OD): δ 7.0-7.3 (m, 16 H), 6.8-7.0 (m, 2H), 4.9-5.0 (m, 1H), 4.75 (d, 1H), 4.1-4.2 (m, 2H). 3.5-4.0 (m, 10H), 2.18-2.3. (m, 1H), 1.6-1.7 (m, 1), 1.47 & 1.41 (2d, 3H), 1.22 (t, 3H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 19.72 & 17.86 ppm.

Diethyl phosphonate 11.13: Compound **11.13** (6 mg) was prepared as described above in Scheme 5 from **11.4** (30 mg, 34 μmol) and triflate phosphonate **5.3** (52 mg, 172 μmol), followed by TFA treatment. NMR ($CDCl_3$ + ~10 %CD_3OD): δ 7.1-7.32 (m, 11 H), 6.9-7.0 (d, 2H), 4.75 (d, 1H), 4.1-4.2 (2q, 4H), 3.84-3.9 (m, 1H), 3.4-3.8 (m, 8H), 2.7-3.1 (m, 4H), 2.1-2.5 (m, 1H), 1.5-1.7 (m, 2H), 1.25-1.35 (2t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 21.63 ppm. MS: 680 (M + 1).

Scheme 12

11.9 →

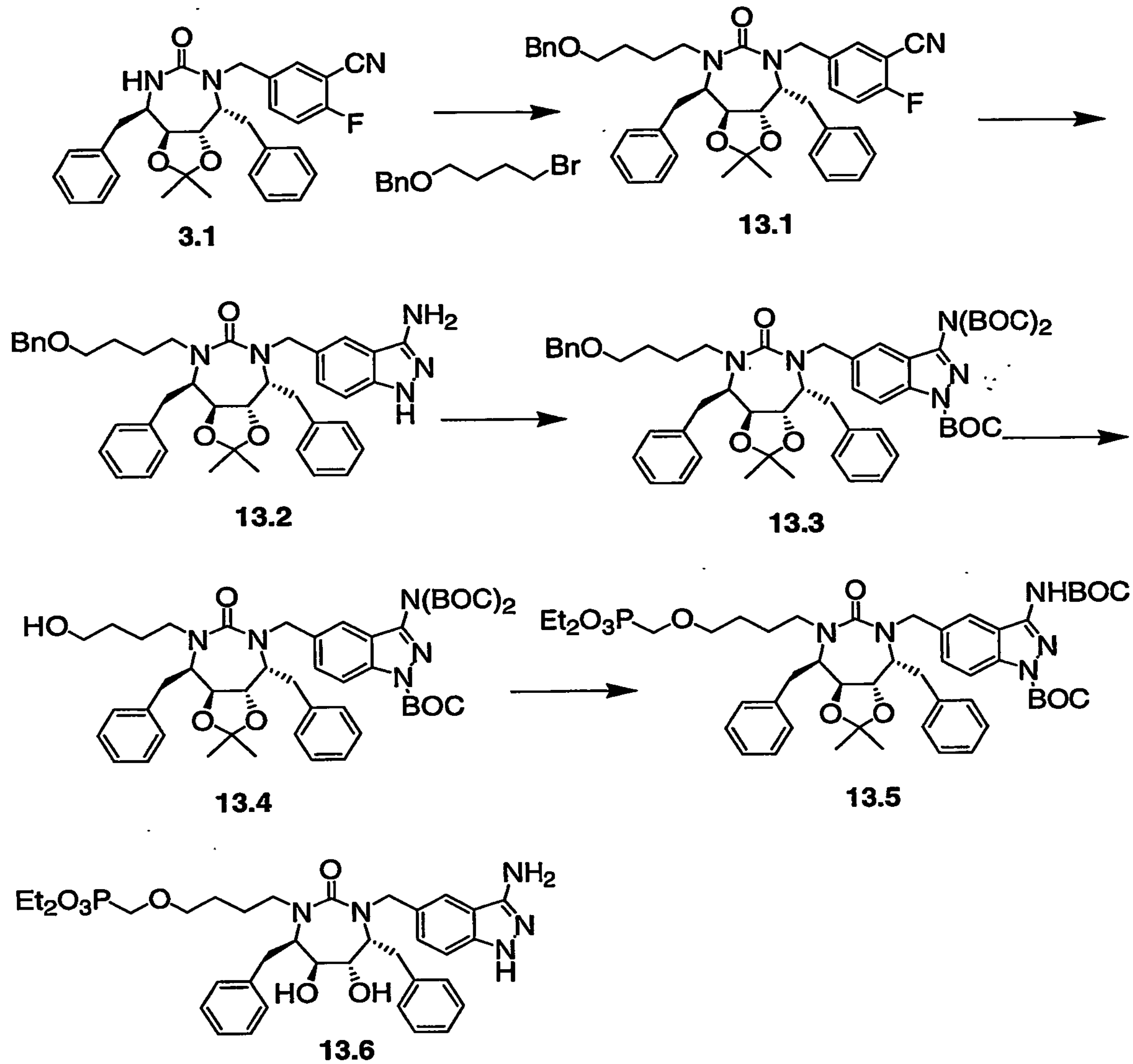


12.1

12.2

Butyl lactate phosphonate 12.2: A pyridine solution (0.3 mL) of **11.9** (27 mg, 22 µmol), butyl lactate (31 mg, 265 µmol), and DCC (28 mg, 132 µmol) was stirred at room temperature for 5 min, then reacted at 70°C for 1.5 h. The reaction mixture was concentrated under reduced pressure, partitioned between methylene chloride and saturated NaCl solution, and purified by preparative TLC to give **12.1** (12 mg). A methylene chloride solution (0.8 mL) of **12.1** (12 mg) was treated with TFA (0.2 mL) for 4 h, concentrate. The residue was purified by preparative TLC to give **12.2** (3 mg, 16 %). NMR ($CDCl_3$ + ~10 %CD_3OD): δ 6.8-7.4 (m, 18H), 6.4-6.6 (m), 4.9-5.05 (m, 1H), 4.75 (d, 1H), 4.1-4.2 (m, 2H). 3.5-4.0 (m, 10H), 3.1-3.25 (m, 2H), 2.2-2.35 (m, 1H), 1.8-1.9 (m, 1H), 1.4 & 1.8 (m, 7H), 1.22 (t, 3H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 19.69 & 17.86 ppm.

Scheme 13



Benzyl ether 13.1: A DMF solution (5 mL) of **3.1** (1 g, 2 mmol) was treated with NaH (0.24 g of 60% oil dispersion, 6 mmol) for 30 min, followed by the addition of sodium iodide (0.3 g, 2 mmol),and benzoxybutyl bromide (0.58 g, 2.4 mmol). After the reaction for 5 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **13.1** (0.58 g, 44 %).

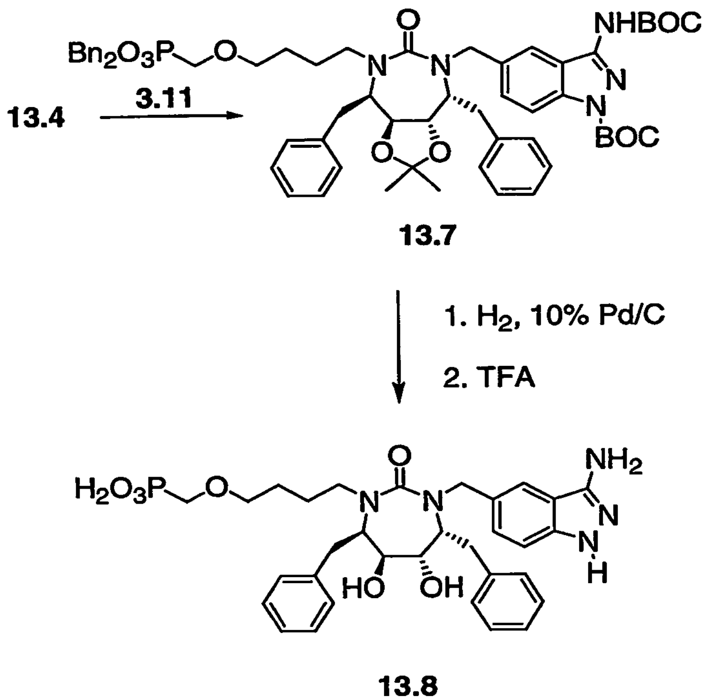
Aminoindazole 13.2: A n-butanol solution (10 mL) of **11.1** (0.58 g, 0.87 mmol) and hydrazine hydrate (0.88 g, 17.5 mmol) was heated at reflux for 4 h. The reaction mixture was concentrated under reduced pressure to give crude **13.2** (0.56 g).

Tri-BOC-aminoindazole 13.3: A methylene chloride solution (10 mL) of **13.2** (0.55 g, 0.82 mmol), DIPEA (0.42 g, 3.2 mmol), $(BOC)_2O$ (0.71 g, 3.2 mmol), and DMAP (0.3 g, 2.4 mmol) was stirred for 4 h at room temperature, partitioned between methylene chloride and 5% citric acid solution, dried, purified by silica gel chromatography to give **13.3** (0.56 g, 71 %, 2 steps).

3-Hydroxybutyl cyclic urea 13.4: An ethyl acetate/methanol solution (30 mL/5 mL) of **11.3** (0.55 g, 0.56 mmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (0.2 g) for 3 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to afford crude **13.4** (0.5 g, 98 %).

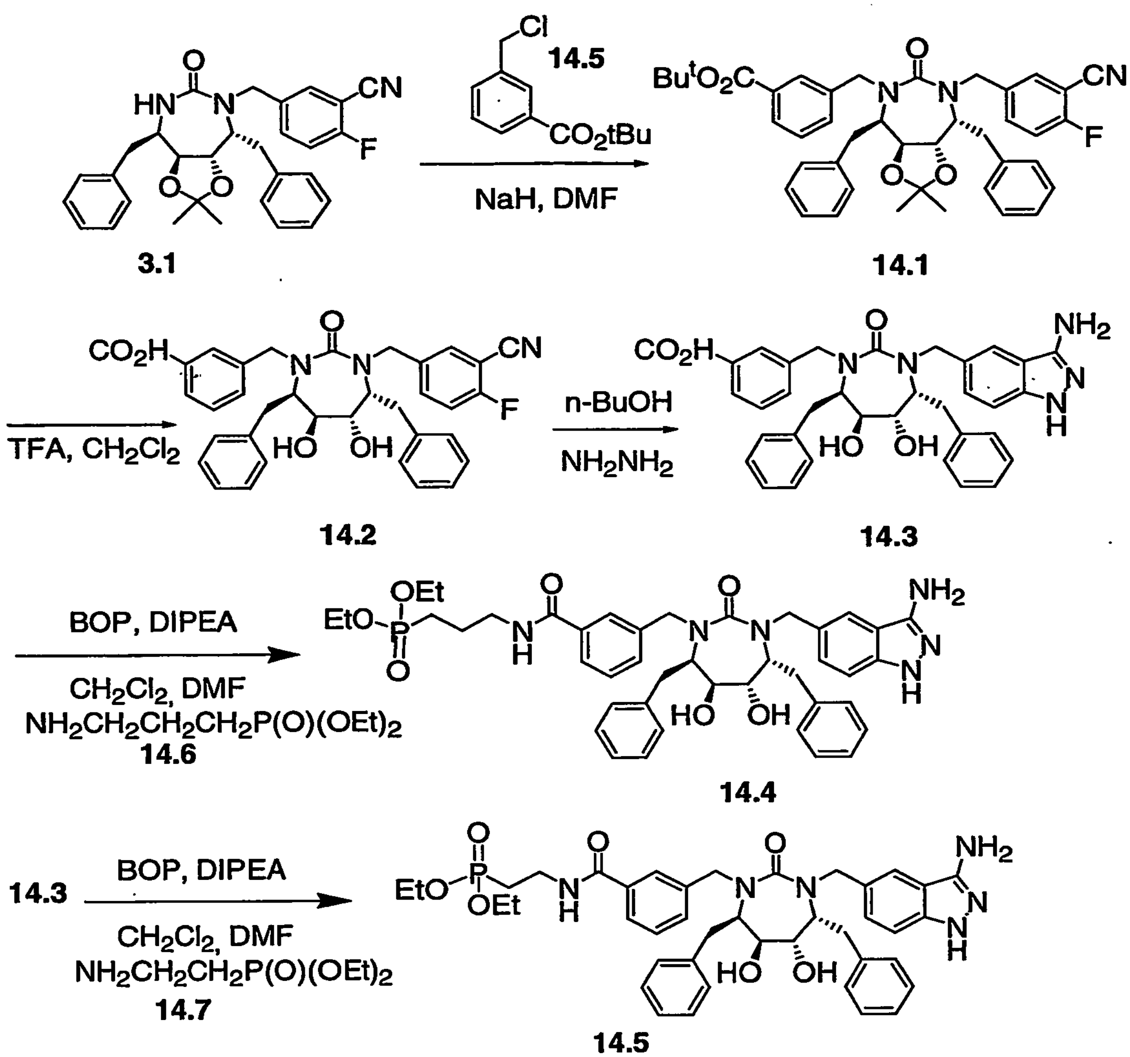
Diethyl phosphonate 13.6: A THF solution (1 mL) of **13.4** (5 mg, 56 μmol) and triflate diethyl phosphonate **5.3** (30 mg, 100 μmol) was cooled to –3°C, followed by addition of n-BuLi (80 μl of 2.5 M hexane solution, 200 μmol). After 2 h reaction, the reaction mixture was partitioned between methylene chloride and saturated NaCl solution, concentrated under reduced pressure to give crude **13.5**. The residue was dissolved in methylene chloride (0.8 mL) and treated with TFA (0.2 mL) for 4 h. concentrated under reduced pressure, and purified by HPLC to give **13.6** (8 mg, 21%). NMR ($CDCl_3$): δ 7.1-7.4 (m, 11H), 7.0-7.1 (m, 2H) 4.81 (d, 1H), 4.1-4.25 (m, 4H). 3.85-3.95 (m, 1H), 3.4-3.8 (m, 7H), 3.3-3.4 (m, 1H), 2.8 - 3.25 (m, 5H), 2.0-2.15 (m, 1H), 1.3-1.85 (m, 10H). P NMR ($CDCl_3$): 21.45 ppm.

Scheme 13a



Phosphonic diacid 13.8: Compound **13.8** (4.5 mg) was prepared from **13.4** as described above for the preparation of **11.7** from **11.4** (Scheme 11). NMR (CD_3OD): δ 7.41 (s, 1H), 7.1-7.4 (m, 10H), 6.9-7.0 (m, 2H) 4.75 (d, 1H), 3.8-4.0 (m, 1H). 3.4-3.8 (m, 8H), 2.8 -3.25 (m, 5H), 2.1-2.25 (m, 1H), 1.6-1.85 (m, 4H). MS: 638 (M + 1).

Scheme 14



t-Butyl ester 14.1: A DMF solution (3 mL) of **3.1** (0.5 g, 1 mmol) was treated with NaH (80 mg of 60% oil dispersion, 2 mmol) for 10 min, followed by the addition of **14.5** (0.25 g, 1.1 mmol). After the reaction for 1 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **14.1** (0.4 g, 59%).

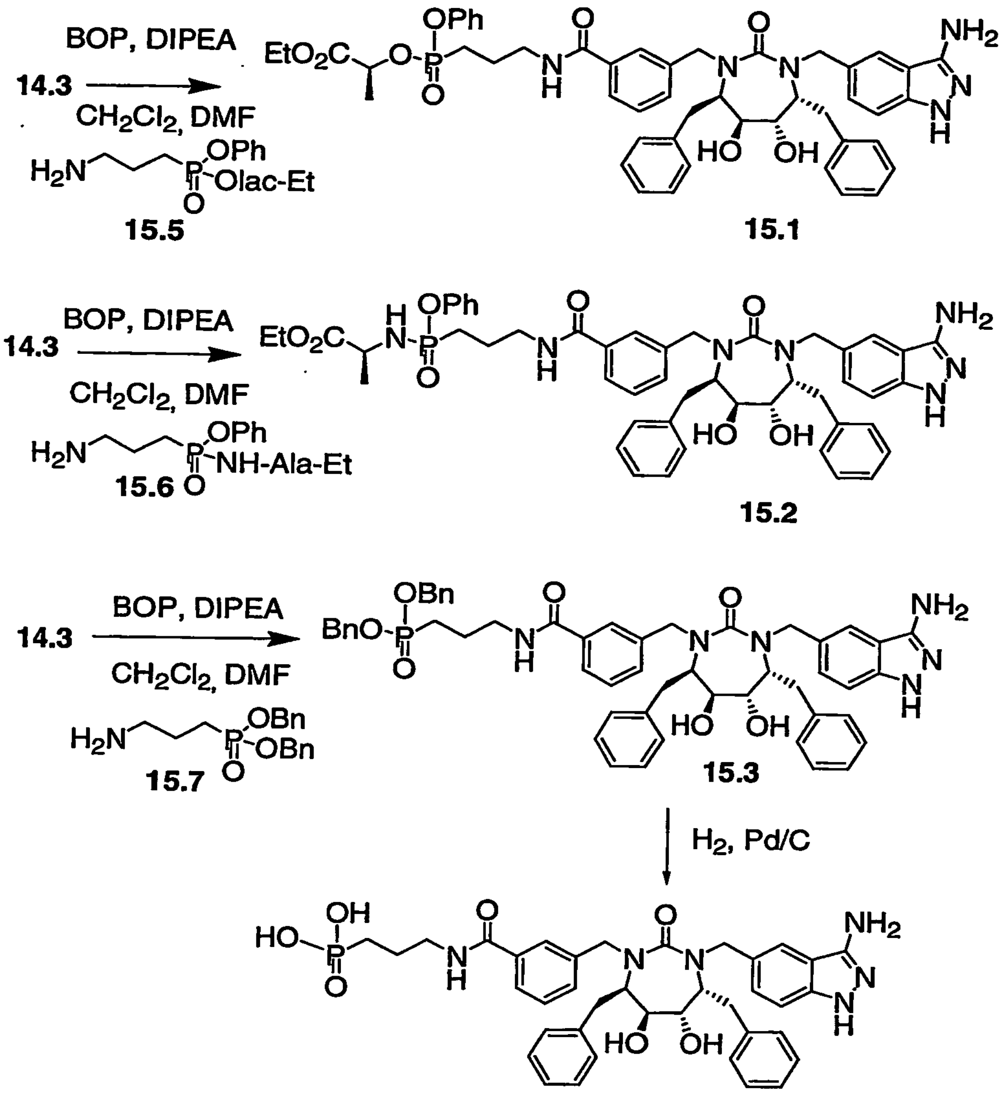
Aminoindazole derivative 14.3: A methylene chloride solution (5 mL) of **14.1** (0.4 g, 0.58 mmol) was treated with TFA (1 mL) at room temperature for 1.5 h, and then concentrated under reduced pressure to give crude **14.2**. The crude **14.2** was dissolved in n-BuOH (5 mL) and reacted with hydrazine hydrate (0.58 g, 11.6 mmol) at reflux for 5 h. The reaction

mixture was concentrated under reduced pressure and purified by silica gel chromatography to give the desired product **14.3** (0.37 g, quantitative yield).

Diethylphosphonate ester 14.4: A methylene chloride solution (3 mL) of **14.3** (23 mg, 38 μmol) was reacted with aminopropyl-diethylphosphonate **14.6** (58 mg, 190 μmol), DIPEA (50 mg, 380 μmol), and ByBOP (21 mg, 48 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **14.4** (9 mg, 34 %). NMR ($CDCl_3$ + ~10 %CD_3O): δ 7.87 (t, 1H), 7.61 (b, 1H), 7.51 (s, 1H), 7.14-7.2 (m, 10 H), 6.93-7.0 (m, 4H), 4.79 (d, 2H), 3.99-4.04 (m, 4H), 3.38-3.65 (m, 6H), 2.60-3.2 (m, 6 H), 1.70-1.87 (m, 4H), 1.25 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 32.7 ppm.

Diethylphosphonate ester 14.5: A methylene chloride solution (2 mL) of **14.3** (13 mg, 21 μmol) was reacted with aminoethyl-diethylphosphonate oxalate **14.7** (23mg, 85 μmol), DIPEA (22 mg, 170 μmol), and ByBOP (12 mg, 25 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give 14.5 (5mg, 30%). Ms: 783 (M + 1). NMR ($CDCl_3$ + ~10 %CD_3O): δ 7.88 (b, 1H), 7.58 (b, 1H), 7.49 (s, 1H), 7.14-7.2 (m, 10 H), 6.90-7.0 (m, 4H), 4.75 (d, 2H), 3.90-4.04 (m, 4H), 2.50-3.3 (m, 6 H), 1.97-2.08 (m, 2H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 30.12 ppm.

Scheme 15



15.4

Monophenol-ethyl lactate phosphonate prodrug 15.1: A methylene chloride/DMF solution (2 mL/0.5 mL) of **14.3** (30 mg, 49 μmol) was reacted with aminopropyl-phenol-ethyl lactate phosphonate **15.5** (100 mg, 233 μmol), DIPEA (64 mg, 495 μmol), and BOP reagent (45 mg, 100 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by silica gel chromatography to give **15.1** (28 mg, 64 %). NMR ($CDCl_3$ + ~10 %CD_3O): δ 7.83 (b, 1H), 7.59 (b, 1H), 7.51 (s, 1H), 7.14-7.2 (m, 11 H), 6.90-7.0 (m, 4H), 4.75-4.87 (d +

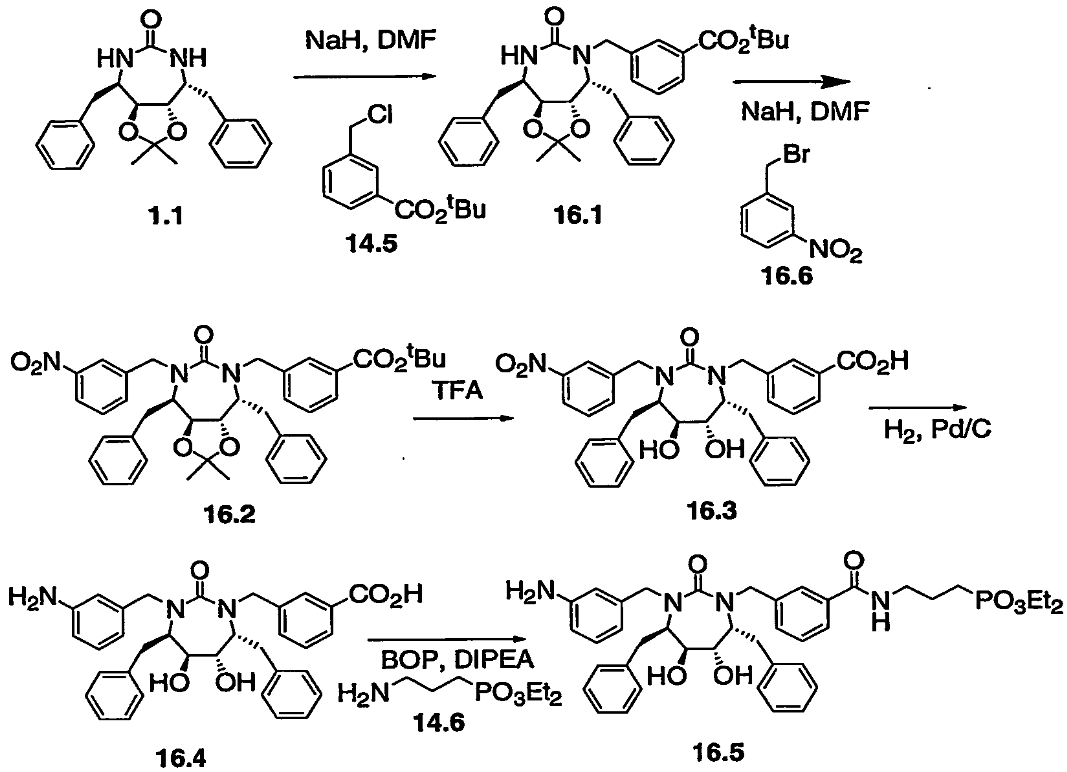
q, 3H), 4.10 (q, 2H), 3.3-3.61 (m, 6H), 2.60-3.2 (m, 6H), 1.92-2.12 (m, 4H), 1.30 (d, 3H), 1.18 (t, 3H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 30.71 ppm. MS: 903 (M + 1).

Phenol-ethyl alanine phosphonate prodrug 15.2: A methylene chloride/DMF solution (2 mL/0.5 mL) of **14.3** (30 mg, 49 µmol) was reacted with aminopropyl-phenol-ethyl alanine phosphonate **15.6** (80 mg TFA salt, 186 µmol), DIPEA (64 mg, 500 µmol), and BOP reagent (45 mg, 100 µmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **15.2** (12 mg, 27 %). NMR ($CDCl_3$ + ~10 %CD_3O): δ 7.91 (b, 1H), 7.61 (b, 1H), 7.52 (s, 1H), 7.14-7.2 (m, 11 H), 6.90-7.0 (m, 4H), 4.75 (d, 2H), 3.82-4.1 (2q, 3H), 3.4-3.65 (m, 6H), 2.60-3.15 (m, 6H), 1.8-2.0 (m, 4H), 1.3 (d, 3H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 32.98 & 33.38 ppm. MS: 902 (M + 1).

Dibenzyl phosphonate 15.3: A methylene chloride/DMF solution (2 mL/0.5 mL) of **14.3** (30 mg, 49 µmol) was reacted with aminopropyl dibenzyl phosphonate **15.7** (86 mg TFA salt, 200 µmol), DIPEA (64 mg, 500 µmol), and BOP reagent (45 mg, 100 µmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **15.3** (20 mg, 44%). NMR ($CDCl_3$ + ~5%CD_3O): δ 7.50-7.58 (m, 2H), 7.14-7.3 (m, 21 H), 6.90-7.0 (m, 4H), 4.7-5.1 (m, 6H), 3.6-3.8 (m, 4H), 3.3-3.55 (m, 2H), 2.60-3.15 (m, 6H), 1.8-2.0 (m, 4H). P NMR ($CDCl_3$ + ~5 %CD_3OD): 33.7 ppm. MS: 907 (M + 1).

Phosphonic diacid 15.4: An ethanol solution (5 mL) of **15.3** (17 mg, 18.7 µmol) was hydrogenated at 1 atm in the presence of 10 % Pd/C for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **15.4** (12 mg, 85%). NMR (CD_3O + 20%$CDCl_3$): δ 7.88 (b, 1H), 7.59 (b, 1H), 7.6 (s, 1H), 7.1-7.25 (m, 10 H), 6.90-7.1 (m, 4H), 4.8 (d, 2H + water peak), 3.6-3.8 (m, 4H), 3.4-3.5 (m, 2H), 1.85-2.0 (m, 4H).

Scheme 16



Monobenzyl derivative 16.1: A DMF solution (4 mL) of **1.1** (0.8 g, 2.2 mmol) was treated with NaH (0.18 g of 60% oil dispersion, 4.4 mmol) for 10 min at room temperature followed by the addition of 14.5 (0.5 g, 2.2 mmol). The resulting solution was reacted at room temperature for 2 h, worked up, and then purified to afford **16.1** (0.48 g, 40%).

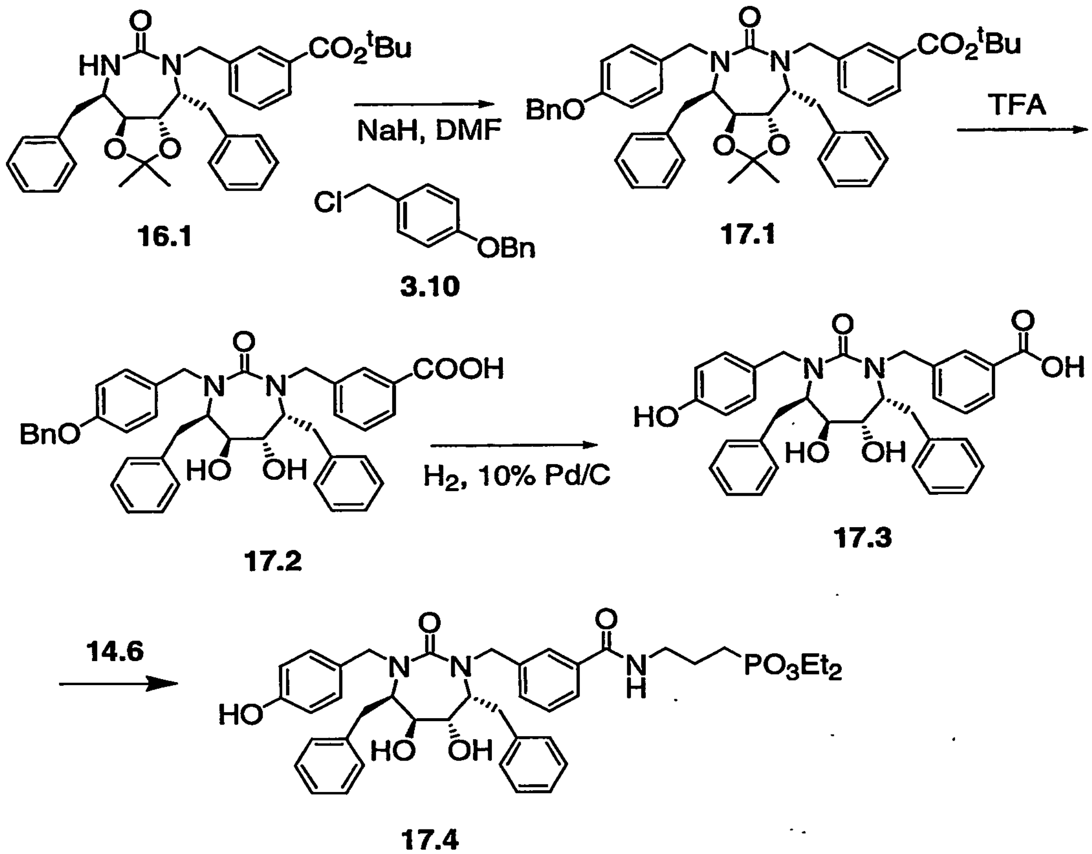
3-Nitrobenzyl cyclic urea derivative 16.2: A DMF solution (0.5 mL) of **16.1** (65 mg, 117 µmol) was treated with NaH (15 mg of 60% oil dispersion, 375 µmol) for 10 min at room temperature, followed by the addition of 3-nitrobenzyl bromide (33 mg, 152 µmol). The resulting solution was reacted at room temperature for 1 h, worked up, and purified by preparative TLC to afford **16.2** (66 mg, 82%).

Diol 16.3: A methylene chloride solution (2 mL) of **16.2** (46 mg, 61 µmol) was treated with TFA (0.4 mL) for 2 h at room temperature, and then concentrated under reduced pressure to afford **16.3**. This material was used without further purification.

3-Aminobenzyl cyclic urea 16.4: An ethyl acetate/ethanol (5 mL/1 mL) solution of **16.3** (crude) was hydrogenated at 1 atm in the presence of 10% Pd/C for 2 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure, and purified by preparative TLC to afford **16.4** (26 mg, 70%, 2 steps).

Diethyl phosphonate 16.5: A methylene chloride/DMF solution (2 mL/0.5 mL) of **16.4** (24 mg, 42 μmol) was reacted with aminopropyl-diethylphosphonate ester TFA salt **14.6** (39 mg, 127 μmol), DIPEA (27 mg, 210 μmol), and BOP reagent (28 mg, 63 μmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give **16.5** (20.7 mg, 63 %). NMR ($CDCl_3$ + ~10 %CD_3O): δ 7.62 (b, 1H), 7.51 (s, 1H), 7.0-7.35 (m, 12 H), 6.95 (d, 2H), 6.85 (d, 2H), 4.6-4.71 (2d, 2H), 3.95-4.1 (m, 4H). 3.3-3.55 (m, 3H), 2.60-2.8 (m, 2H), 2.95-.3. 15 (m, 4 H), 1.85-2.0 (m, 4H), 1.25 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 32.65 ppm.

Scheme 17

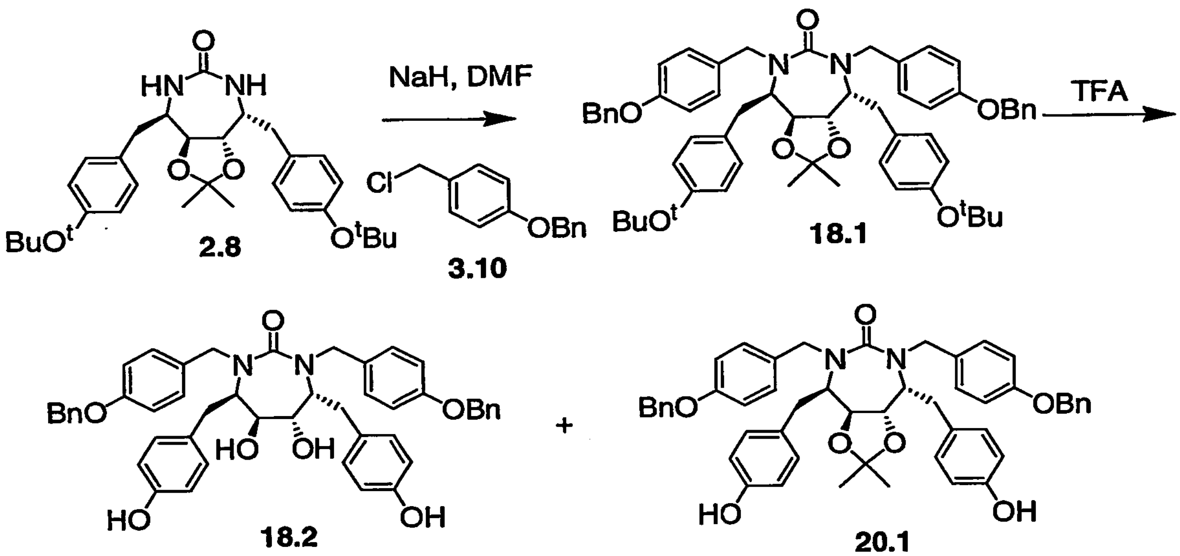


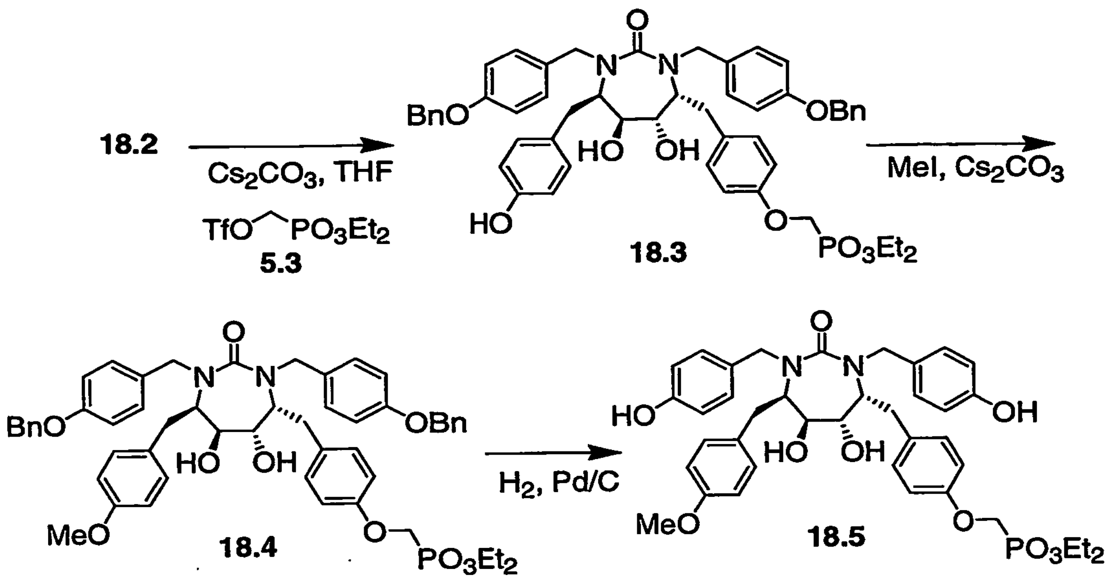
p-Benzoxybenzyl cyclic urea derivative 17.1: A DMF solution (0.5 mL) of **16.1** (65 mg, 117 µmol) was treated with NaH (15 mg of 60% oil dispersion, 375 µmol) for 10 min at room temperature, followed by the addition of 4-benzoxy benzyl chloride **3.10** (35 mg, µmol). The resulting solution was stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure, purified by preparative TLC to generate **17.1** (62 mg, 70%).

Diethyl phosphonate 17.3: A methylene chloride solution (2 mL) of **17.1** (46 mg, 61 µmol) was treated with TFA (0.4 mL) for 2 h at room temperature, and then concentrated under reduced pressure to give crude **17.2**. An ethyl acetate/ethanol solution (3 mL/2 mL) of the crude **17.2** was then hydrogenated at 1 atm in the presence of 10% Pd/C (10 mg) for 5 h at room temperature. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to afford **17.3** (crude).

Diethyl phosphonate cyclic urea 17.4: A methylene chloride/DMF solution (2 mL/0.5 mL) of **17.3** (25 mg, 42 µmol) was reacted with aminopropyl-diethylphosphonate ester TFA salt **14.6** (40 mg, 127 µmol), DIPEA (27 mg, 210 µmol), and BOP reagent (28 mg, 63 µmol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give **17.4** (14.6 mg, 44 %). NMR ($CDCl_3$ + ~10 %CD_3O): δ 7.82 (t), 7.62 (d, 1H), 7.51 (s, 1H), 7.05-7.35 (m, 10 H), 6.8-6.95 (2d, 4H), 6.85 (d, 2H), 4.8 (d, 1H), 4.65 (d, 1H), 3.95-4.1 (m, 4H). 3.4-3.75 (m, 6H), 2.60-3.2 (m), 1.85-2.0 (m, 4H), 1.25 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 32.72 ppm.

Scheme 18





Dibenzyl derivative 18.1: A DMF solution (3 mL) of compound **2.8** (0.4 g, 0.78 mmol) was reacted with 60%NaH (0.13 g, 1.96 mmol), 4-benzoxy benzylchloride **3.10** (0.46 g, 1.96 mmol) and sodium iodide (60 mg, 0.39 mmol) at room temperature for 4 h. The reaction mixture was partitioned between methylene chloride and saturated $NaHCO_3$ solution. The organic phase was isolated, dried over Na_2SO_4, concentrated under reduced pressure, and purified by silica gel chromatography to give the desired product **18.1** (0.57 g, 81%).

Diol derivative 18.2 and diphenol derivative 20.1: A methylene chloride solution (4 mL) of **18.1** (0.57 g, 0.63 mmol) was treated with TFA (1 mL) at room temperature for 20 min, concentrated under reduced pressure, and purified by silica gel chromatography to give diol derivative **18.2** (133 mg, 28 %) and diphenol derivative **20.1** (288 mg. 57.6%).

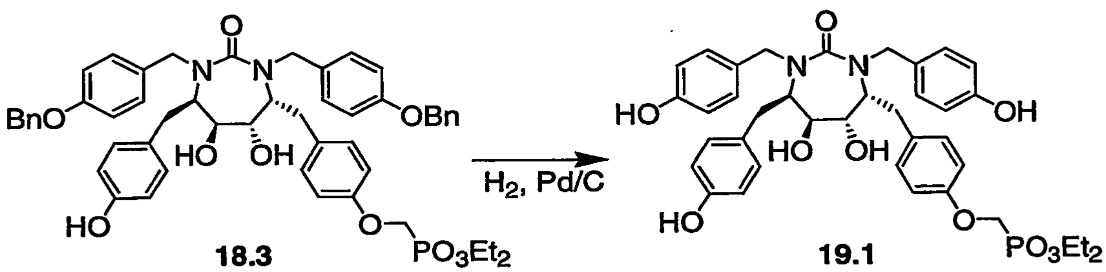
Monophosphonate derivative 18.3: A THF solution (10 mL) of **18.2** (130 mg, 0.17 mmol) was stirred with cesium carbonate (70 mg, 0.21 mmol) and diethylphosphonate triflate **5.3** (52 mg, , 0.17 mmol) at room temperature for 4 h.. The reaction mixture was concentrated under reduced pressure and purified to give **18.3** (64 mg, 41 %), and recovered **18.2** (25 mg, 19%).

Methoxy derivative 18.4: A THF solution (2 mL) of **18.3** (28 mg, 25 μmol) was treated with cesium carbonate (25 mg, 76 μmol) and iodomethane (10 eq. Excess) at room

temperature for 5 h. The reaction mixture was concentrated under reduced pressure and partitioned between methylene chloride and saturated $NaHCO_3$. The organic phase was separated, concentrated under reduced pressure and the residue purified by preparative TLC to afford **18.4** (22 mg, 78%).

Diethylphosphonate 18.5 : An ethyl acetate/ethanol (2 mL/2 mL) solution of **18.4** (22 mg, 24 μmol) was hydrogenated at 1 atm in the presence of 10% Pd/C for 3 h. The catalyst was removed by filtration, the filtrate was concentrated under reduced pressure to give the desired product **18.5** (18 mg, quantitative). NMR ($CDCl_3$ + ~10 %CD_3O): δ 6.7-7.0 (m, 12 H), 6.62-6.69 (m, 4H), 4.65 (d, 1H), 4.50 (d, 1H), 4.18-4.3 (m, 6H). 3.75 (s, 3H), 3.3-3.4 (m, 4H), 2.8-3.0 (m, 6H), 1.30 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 20.16 ppm.

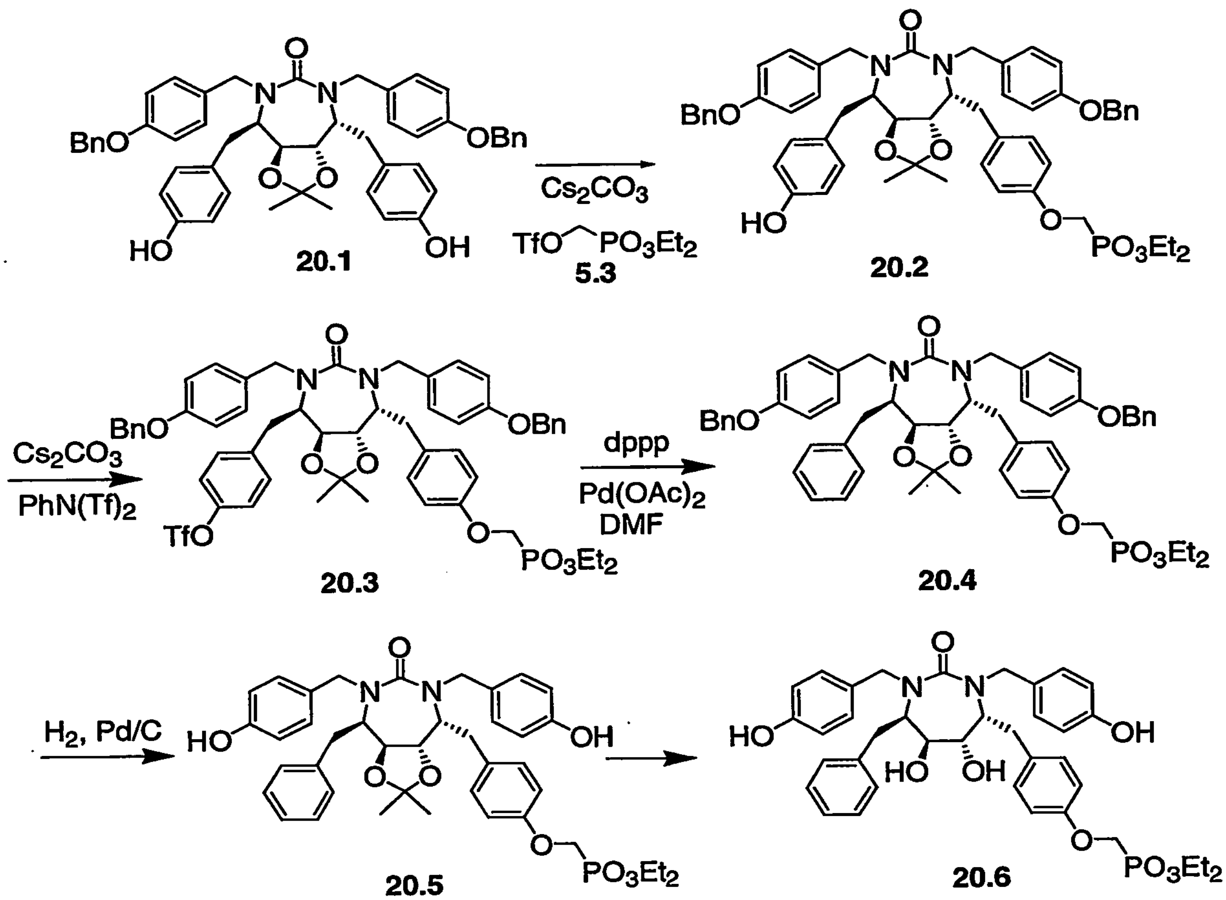
Scheme 19



18.3 19.1

Diethyl phosphonate 19.1: An ethyl acetate/ethanol (2 mL/1 mL) solution of **18.3** (14 mg, 15.5 μmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (5 mg) for 3 h. The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **19.1** (10 mg, 90%). NMR ($CDCl_3$ + ~15 %CD_3O): δ 6.6-7.0 (m, 16 H), 4.5-4.65 (2d, 2H), 4.1-4.3 (m, 6H). 2.7-3.0 (m, 6H), 1.29 (t, 6H). P NMR ($CDCl_3$ + ~15 %CD_3OD): 20.12 ppm.

Scheme 20



Monophosphonate 20.2: A THF solution (8 mL) of **20.1** (280 mg, 0.36 mmol) was stirred with cesium carbonate (140 mg, 0.43 mmol) and diethylphosphonate triflate **5.3** (110 mg, 0.36 mmol) at room temperature for 4 h.. The reaction mixture was concentrated under reduced pressure and purified to give **20.2** (130mg, 39%), and recovered **20.1** (76 mg, 27%).

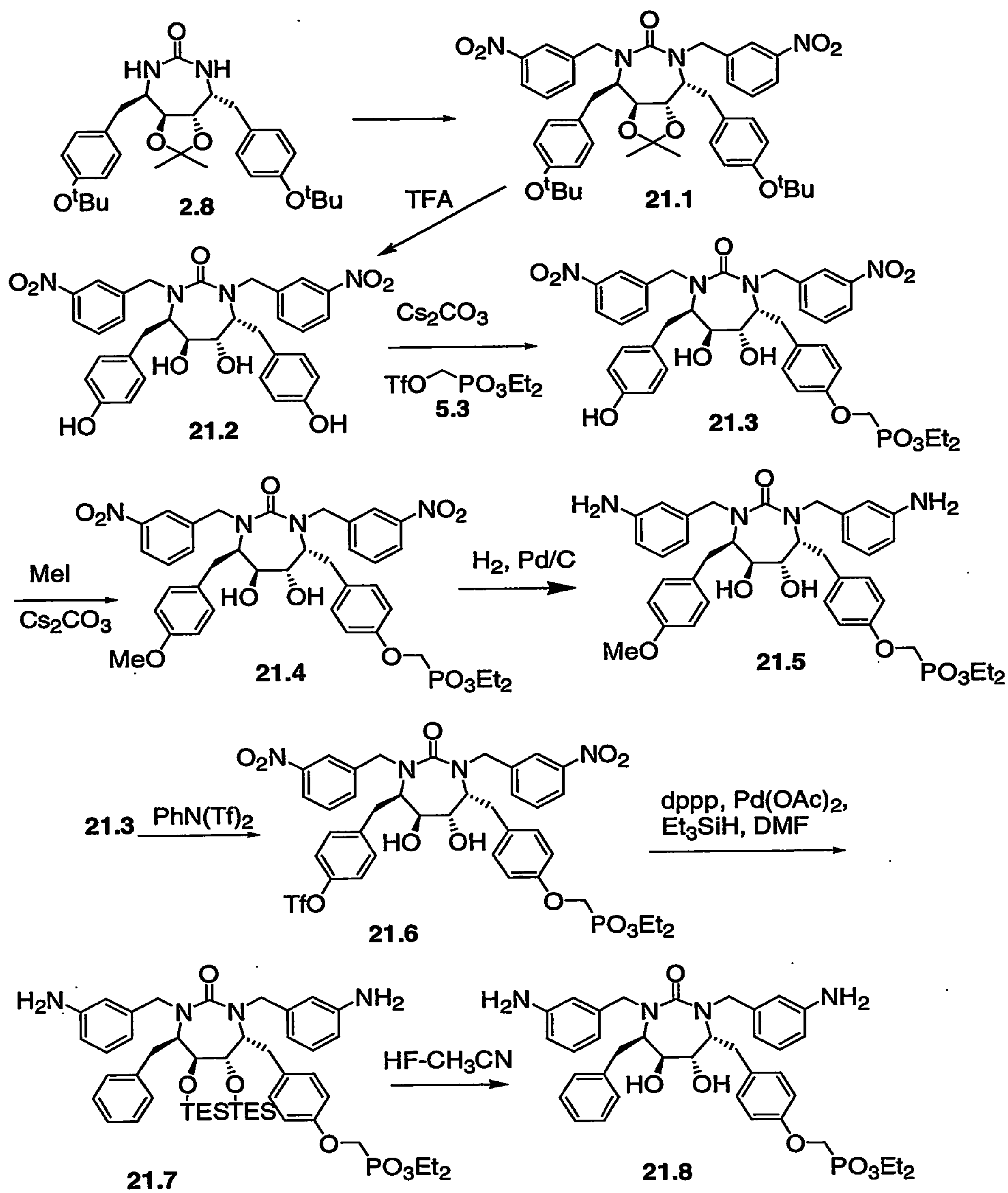
Triflate derivative 20.3: A THF solution (6 mL) of **20.2** (130 mg, 0.13 mmol) was stirred with cesium carbonate (67 mg, 0.21 mmol) and N-phenyltrifluoromethane-sulfonimide (60mg, 0.17 mmol) at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and purified to give **20.3** (125 mg, 84%).

Benzyl ether 20.4: To a DMF solution (2 mL) of $Pd(OAc)_2$ (60 mg, 267 μmol), and dppp (105 mg. 254 μmol) was added **20.3** (120 mg, 111 μmol) under nitrogen, followed by the addition of triethylsilane (0.3 mL). The resulting solution was stirred at room temperature for

4 h, then concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford **20.4** (94 mg, 92%).

Diethyl phosphonate 20.6: An ethyl acetate/ethanol (2 mL/2 mL) solution of **20.4** (28 mg, 30 μmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (5 mg) for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **20.5**. The crude product **20. 5** was redissolved in methylene chloride (2 mL) and treated with TFA (0.4 mL) and a drop of water. After 1 h stirring at room temperature, the reaction mixture was concentrated under reduced pressure, and purified by preparative TLC plate to give **20.6** (18 mg, 85 %, 2 steps). δ 6.6-7.3 (m, 17 H), 4.65 (d, 1H), 4.58 (d, 1H), 4.18-4.3 (m, 6H), 3.3-3.5 (m, 4H), 2.8-3.1 (m), 1.34 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 20.16 ppm. MS: 705 (M + 1).

Scheme 21



Bis-(3-nitrobenzyl) derivative 21.1: A DMF solution (2 mL) of compound **2.8** (0.3 g, 0.59 mmol) was reacted with 60%NaH (0.07 g, 1.76 mmol), 3-nitrobenzyl bromide (0.38 g, 1.76 mmol) and sodium iodide (60 mg, 0.39 mmol) at room temperature for 3 h. The reaction

mixture was partitioned between methylene chloride and saturated $NaHCO_3$ solution. The organic phase was isolated, dried over Na_2SO_4, concentrated under reduced pressure, and purified by silica gel chromatography to give the desired product **21.1** (0.37 g, 82%).

Diphenol derivative 21.2: A methylene chloride solution (4 mL) of **21.1** (0.37 g, 0.47 mmol) was treated with TFA (1 mL) at room temperature for 3 h, and then concentrated under reduced pressure, and azeotroped with CH_3CN twice to give diphenol derivative **21.2** (0.3 g, quantitative).

Monophosphonate derivative 21.3: A THF solution (8 mL) of **18.2** (0.28g, 0.44 mmol) was stirred with cesium carbonate (0.17 g, 0.53 mmol) and diethylphosphonate triflate **5.3** (0.14 g, 0.44 mmol) at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and purified to give **21.3** (120 mg, 35%), and recovered **21.2** (150 mg, 53%).

Methoxy derivative 21.4: A THF solution (2 mL) of **21.3** (9 mg, 11 μmol) was treated with cesium carbonate (15 mg, 46 μmol) and iodomethane (10 eq. Excess) at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure and partitioned between methylene chloride and saturated $NaHCO_3$. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by preparative TLC to afford **21.4** (9 mg)

Diethylphosphonate 21.5: A ethyl acetate/ethanol (2 mL/0.5 mL) solution of **21.4** (9 mg, 11 μmol) was hydrogenated at 1 atm in the presence of 10% Pd/C for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product 21.5 (4.3 mg, 49%, 2 steps). NMR ($CDCl_3$ + ~10 %CD_3O): δ 7.0-7.10 (m, 6 H), 6.8-6.95 (m, 4H), 6.5-6.6 (m, 4H), 6.4-6.45 (m, 2H), 4.72 (d, 2H), 4.18-4.3 (m, 6H). 3.72 (s, 3H), 3.4-3.5 (m, 4H), 2.8-3.0 (m, 6H), 1.34 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 19.93 ppm.

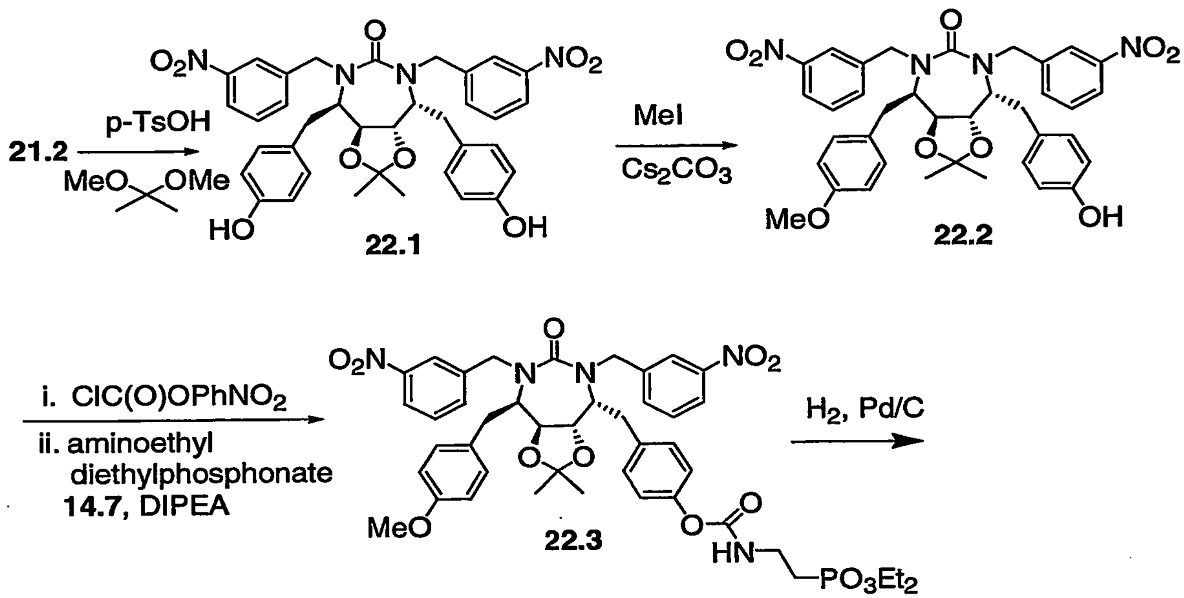
Triflate 21.6: A THF solution (6 mL) of **21.3** (0.1g, 0.14 mmol), cesium carbonate (0.07 g, 0.21 mmol), and N-phenyltrifluoromethane-sulfonimide (60mg, 0.17 mmol) was stirred at

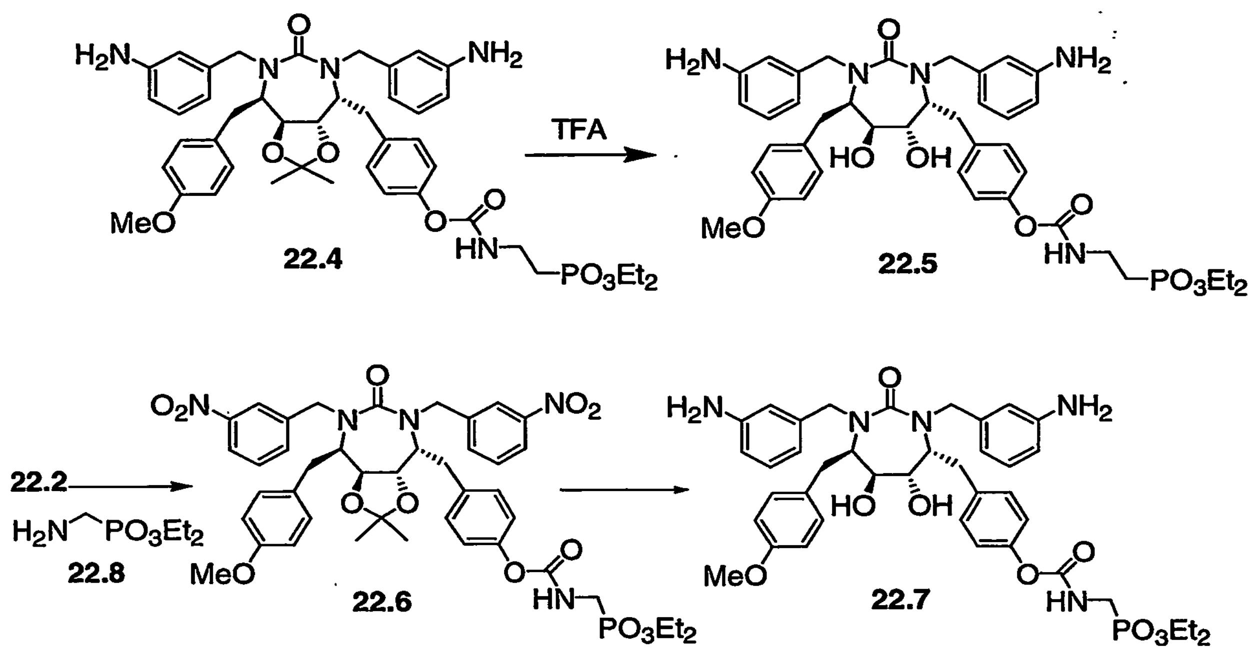
room temperature for 4 h, and then concentrated under reduced pressure, and worked up. The residue was purified by silica gel chromatography to give **21.6** (116 mg, 90%).

Diamine 21.7: A DMF solution (2 mL) of **21.6** (116 mg, 127 μmol), dppp (60 mg, 145 μmol), and $Pd(OAc)_2$ (30 mg, 134 μmol) was stirred under nitrogen, followed by addition of triethylsilane (0.3 mL), and reacted for 4 h at room temperature. The reaction mixture was worked up and purified to give **21.7** (50 mg).

Diethyl phosphonate 21.8: An acetonitrile solution (1 mL) of crude **21.7** (50 mg) was treated with 48% HF (0.1 mL) for 4 h. The reaction mixture was concentrated under reduced pressure, and purified to give **21.8** (10 mg, 11% (2 steps). NMR ($CDCl_3$ + ~10%CD_3O): δ 7.05-7.30 (m, 9 H), 6.8-6.95 (d, 2H), 6.4-6.6 (m, 6H), 4.72 (d, 2H), 4.18-4.3 (m, 6H). 3.4-3.5 (m, 4H), 2.8-3.0 (m, 6H), 1.34 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 19.83 ppm.

Scheme 22





Acetonide 22.1: An acetone/2,2-diemethoxypropane solution (15 mL/5 mL) of compound **21.2** (240 mg, 0.38 mmol) and pyridinium toluenesulfonate (10 mg) was heated at reflux for 30 min. After cooled to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between methylene chloride and saturated $NaHCO_3$ aqueous solution, dried, concentrated under reduced pressure and purified to afford **22.1** (225 mg, 88%).

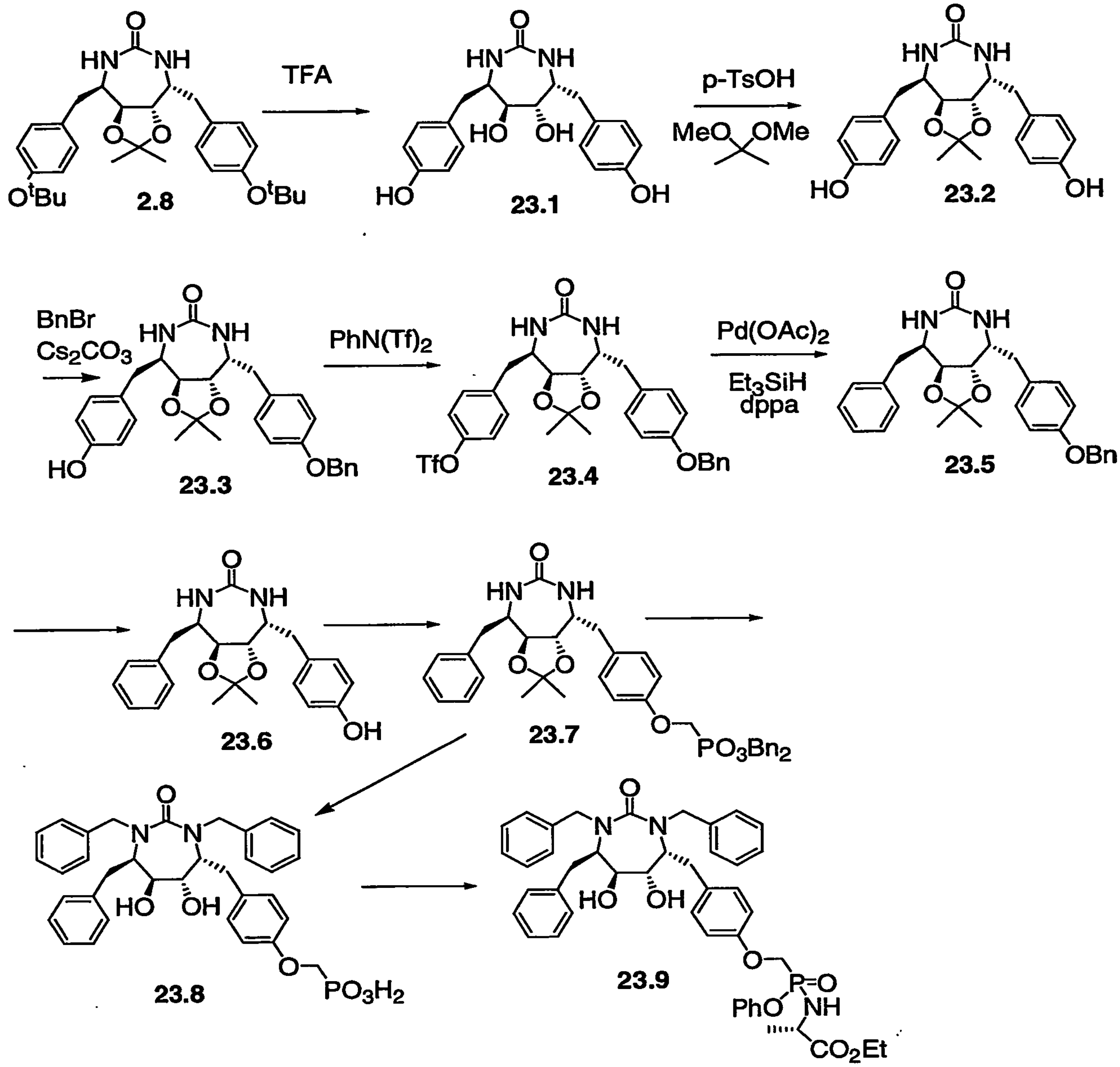
Monomethoxy derivative 22.2: A THF solution (10 mL) of **22.1** (225 mg, 0.33 mmol) was treated with cesium carbonate (160 mg, 0.5 mmol) and iodomethane (52 mg. 0.37 mmol) at room temperature overnight. The reaction mixture was concentrated under reduced pressure, and purified by preparative silica gel column chomatography to afford **22.2** (66 mg, 29%) and recovered starting material **22.1** (25 mg, 11%).

Diethyl phosphonate 22.3: A methylene chloride solution (2 mL) of **22.2** (22 mg, 32 µmol), DIPEA (9 mg, 66 µmol), and p-nitrophenyl chloroformate (8 mg, 40 µmol) was stirred at room temperature for 30 min. The resulting reaction mixture was reacted with DIPEA (10 mg, 77 µmol), and aminoethyl diethylphosphonate **14.7** (12 mg. 45 µmol) at room temperature overnight. The reaction mixture was washed with 5% citric acid solution, saturated $NaHCO_3$, dried, and purified by preparative TLC to afford **22.3** (12 mg, 43%).

Bis(3-aminobenzyl)-diethylphosphonate ester 22.5: An ethyl acetate/t-BuOH (4 mL/2 mL) solution of **22.3** (12 mg, 13 μmol) was hydrogenated at 1 atm in the presence of 10% Pd/C 95 mg) at room temperature for 5 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure, and purified by preparative TLC to give **22.4** (8 mg, 72%). A methylene chloride solution (0.5 mL) of **22.4** (8 mg) was treated with TFA (0.1 mL) at room temperature for 1 h., concentrated under reduced pressure, and then azeotroped with CH_3CN twice to afford **22.5** (8.1 mg, 81%). NMR ($CDCl_3$ + ~10 %CD_3OD): δ 7.2 (d, 1H), 6.95-7.15 (m, 6H), 6.75-6.9 (m, 5 H), 4.66 (d, 1H), 4.46 (d, 1H), 4.06-4.15 (m, 4H). 3.75 (s, 3H), 3.6-3.7 (m, 4H), 2.6-3.1 (m, 6H), 2.0-2.1 (m, 2H), 1.30 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 29.53 ppm. MS: 790 (M + 1).

Bis(3-aminobenzyl) diethylphosphonate ester 22.7: Compound **22.7** was prepared from **22.2** (22 mg, 32 μmol) and aminomethyl diethylphosphonate **22.8** as shown above for the preparation of **22.5** from **22.2.** NMR ($CDCl_3$ + ~10 %CD_3OD): δ 7.24 (d, 1H), 6.8-7.12 (m, 11H), 4.66 (d, 1H), 4.45 (d, 1H), 4.06-4.15 (m, 4H). 3.75 (s, 3H), 2.6-3.1 (m, 6H), 1.30 (t, 6H). P NMR ($CDCl_3$ + ~10 %CD_3OD): 22.75 ppm. MS: 776 (M + 1).

Scheme 23



Diol 23.1: To a solution of compound **2.8** (2.98 g, 5.84 mmol) in methylene chloride (14 mL) was added TFA (6 mL). The resulted mixture was stirred at room temperature for 2 h. Methanol (5 mL) and additional TFA (5 mL) were added. The reaction mixture was stirred for additional 4 h and then concentrated under reduced pressure. The residue was washed with hexane/ethyl acetate (1:1) and dried to afford compound **23.1** (1.8 g, 86%) as an off-white solid.

Benzyl ether 23.3: To a solution of compound **23.1** (1.8 g, 5.03 mmol) in DMF (6 mL) and 2,2-dimethoxyl propane (12 mL) was added p-toluenesulfonic acid monohydrate (0.095 g, 0.5 mmol). The resultant mixture was stirred at 65°C for 3 h. The excess 2,2-dimethoxyl propane was slowly distilled. The reaction mixture was cooled to room temperature and charged with THF (50 mL), benzyl bromide (0.8 mL, 6.73 mmol) and cesium carbonate (2.0 g, 6.13 mmol). The resulted mixture was stirred at 65°C for 16 h. The reaction was quenched with acetic acid aqueous solution (4%, 100 mL) at 0°C, and extracted with ethyl acetate. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford desired mono protected compound **23.3** (1.21 g, 49%).

Benzyl ether 23.5: To a solution of compound **23.3** (0.65 g, 1.33 mmol) and N-phenyltrifluoromethanesulfonimide (0.715 g, 2 mmol) in THF (12 mL) was added cesium carbonate (0.65 g, 2 mmol). The mixture was stirred at room temperature for 3 h. The reaction mixture was filtered through a pad of silica gel and concentrated under reduced pressure. The residue was purified on silica gel chromatography to give triflate **23.4** (0.85 g). To a solution of 1,3-bis(diphenylphosphino)propane (0.275g, 0.66 mmol) in DMF (10 mL) was added palladium(II) acetate (0.15 g, 0.66 mmol) under argon. This mixture was stirred for 2 min. and then added to triflate **23.4**. After stirring for 2 min., triethylsilane was added and the resulted mixture was stirred for 1.5 h. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel to afford compound **23.5** (0.56 g, 89%).

Phenol 23.6: A solution of **23.5** (0.28 g, 0.593 mmol) in ethyl acetate (5 mL) and isopropyl alcohol (5 mL) was treated with 10% Pd/C (0.05g) and stirred under a hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **23.6** (0.22 g, 97%) as a white solid.

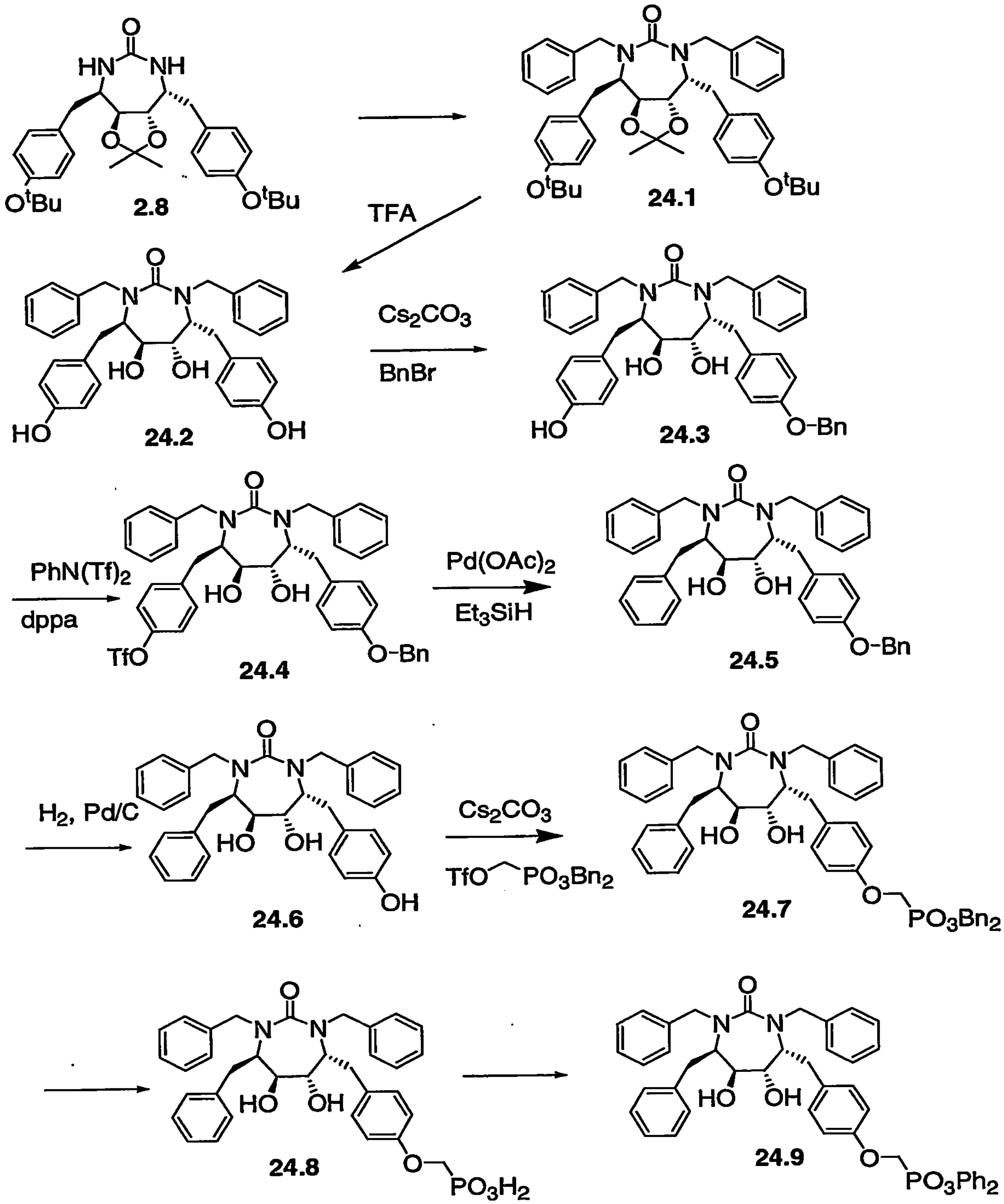
Dibenzyl phosphonate 23.7: To a solution of compound **23.6** (0.215 g, 0.563 mmol) in THF (10 mL) was added dibenzyl triflate **3.11** (0.315 g, 0.74 mmol) and cesium carbonate (0.325g, 1 mmol). The mixture was stirred at room temperature for 2 h, then diluted with ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate, filtered and

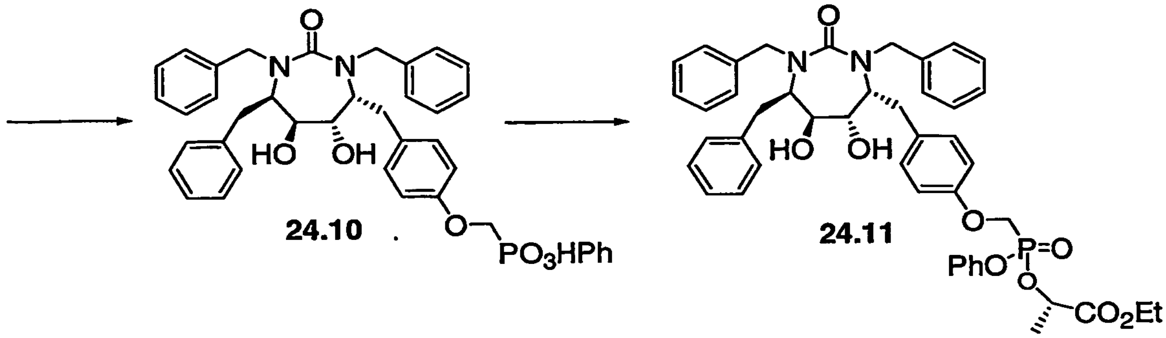
concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **23.7** (0.31 g, 84%).

Diphenyl ester 23.8: A solution of compound **23.7** (0.3 g, 0.457 mmol) and benzyl bromide (0.165 mL, 1.39 mmol) in THF (10 mL) was treated with potassium *tert*-butoxide (1M/THF, 1.2 mL) for 0.5 h. The mixture was diluted with ethyl acetate and washed with HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and treated with 10% Pd/C (0.05 g) under hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was treated with TFA (1 mL) in methanol (5 mL) for 1 h, and then concentrated under reduced pressure. The residue was dissolved in pyridine (1 mL) and mixed with phenol (0.45 g, 4.8 mmol) and 1,3-dicyclohexylcarbodiimide (0.38 g, 1.85 mmol). The mixture was stirred at 70°C for 2 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by chromatography on silica gel to afford compound **23.8** (0.085 g, 24%).

Mono amidate 23.9: To a solution of **23.8** (0.085g, 0.11 mmol) in acetonitrile (1 mL) was added sodium hydroxide (1N, 0.25 mL) at 0°C. After stirred at 0°C for 1 h, the mixture was acidified with Dowex resin to pH = 3, and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in pyridine (0.5 mL) and mixed with L-alanine ethyl ester hydrochloride (0.062 g, 0.4 mmol) and 1,3-dicyclohexyl-carbodiimide (0.125 g, 0.6 mmol). The mixture was stirred at 60°C for 0.5 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by HPLC (C-18, 65% acetonitrile / water) to afford compound **23.9** (0.02 g, 23%). ^{1}H NMR (CDCl3): δ 1.2 (m, 3H), 1.4 (m, 3H), 1.8 (brs, 2H), 2.8-3.1 (m, 6H), 3.5-3.7 (m, 4H), 3.78 (m, 1H), 4.0-4.18 (m, 2H), 4.2-4.4 (m, 3H), 4.9 (m, 2H), 6.8-7.4 (m, 24H). 31P NMR (CDCl3): d 20.9, 19.8. MS: 792 (M+1).

Scheme 24


2.8
24.1
TFA
24.2
Cs_2CO_3
BnBr
24.3
$PhN(Tf)_2$
dppa
24.4
$Pd(OAc)_2$
Et_3SiH
24.5
H_2, Pd/C
24.6
Cs_2CO_3
TfO PO3Bn2
24.7
24.8
24.9



Di-tert butyl ether 24.1: To a solution of compound **2.8** (0.51 g, 1 mmol) and benzyl bromide (0.43g, 2.5 mmol) in THF (6 mL) was added potassium *tert*-butoxide (1M/THF, 2.5 mL). The mixture was stirred at room temperature for 0.5 h, then diluted with ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **24.1** (0.62 g, 90%).

Diol 24.2: To a solution of compound **24.1** (0.62 g, 0.9 mmol) in methylene chloride (4 mL) was added TFA (1 mL) and water (0.1 mL). The mixture was stirred for 2 h, and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **24.2** (0.443g, 92%).

Benzyl ether 24.3: Compound **24.3** was prepared in 46% yield according to the procedure described in Scheme 23 for the preparation of **23.3**.

Triflate 24.4: Compound **24.4** was prepared in 95% yield according to the procedure described in Scheme 23 for the preparation of **23.4**.

Benzyl ether 24.5: Compound **24.5** was prepared in 93% yield according to the procedure described in Scheme 23 for the preparation of **23.5**.

Phenol 24.6: Compound **24.6** was prepared in 96% yield according to the procedure described in Scheme 23 for the preparation of **23.6** from **23.5**.

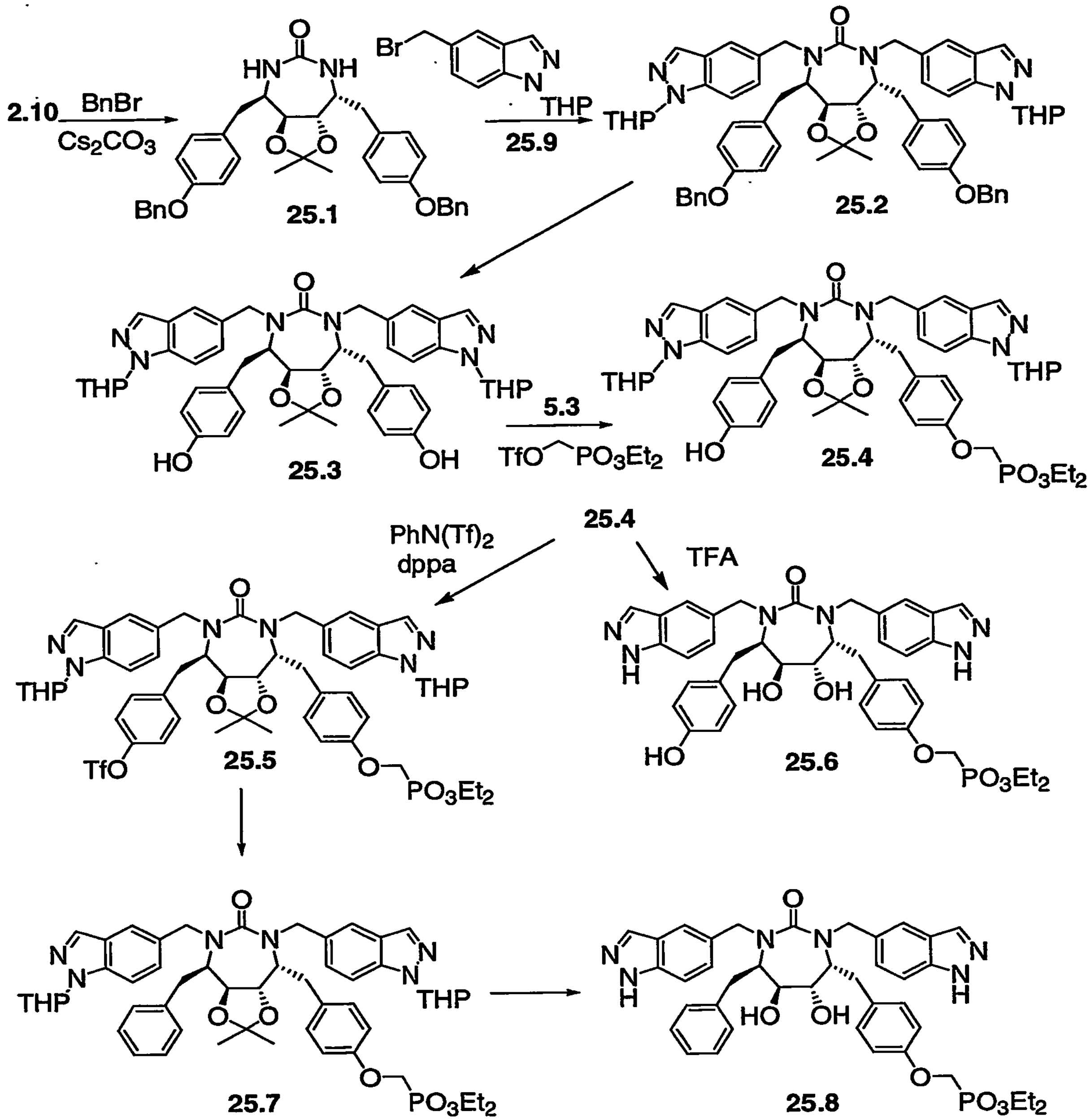
Dibenzyl phosphonate 24.7: Compound **24.7** was prepared in 82% yield according to the procedure described in Scheme 23 for the preparation of **23.7**.

Diacid 24.8: A solution of **24.7** (0.16 g, 0.207 mmol) in ethyl acetate (4 mL) and isopropyl alcohol (4 mL) was treated with 10% Pd/C (0.05g) and stirred under a hydrogen atmosphere (balloon) for 4 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **24.8** (0.125 g, 98%) as a white solid.

Diphenyl ester 24.9: To a solution of compound **24.8** (0.12 g, 0.195 mmol) in pyridine (1 mL) was added phenol (0.19 g, 2 mmol) and 1,3-dicyclohexylcarbodiimide (0.206 g, 1 mmol). The mixture was stirred at 70°C for 2 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by chromatography on silica gel to afford compound **24.9** (0.038 g, 25%).

Mono lactate 24.11: Compound **24.9** was converted, via compound **24.10**, into compound **24.11** in 36% yield according to the procedure described in Scheme 23 for the preparation of **23.9** except utilizing the ethyl lactate ester in place of L-alanine ethyl ester. ^{1}H NMR (CDCl3): δ 1.05 (t, J = 8 Hz, 1.5H), 1.1 (t, J = 8 Hz, 1.5H), 1.45 (d, J = 8 Hz, 1.5H), 1.55 (d, J = 8 Hz, 1.5H), 2.6 (brs, 2H), 2.9-3.1 (m, 6H), 3.5-3.65 (m, 4H), 4.15-4.25 (m, 2H), 4.4-4.62 (m, 2H), 4.9 (m, 2H), 5.2 (m, 1H), 6.9-7.4 (m, 24H). 31P NMR (CDCl3): d 17.6, 15.5. MS: 793 (M+1).

Scheme 25



Dibenzyl ether 25.1: The protection reaction of compound **2.10** with benzyl bromide was carried out in the same manner as described in Scheme 23 to afford compound **25.1**.

Bis indazole 25.2: The alkylation of compound **25.1** with bromide **25.9** was carried out in the same manner as described in Scheme 23 to afford compound **25.2** in 96% yield.

Diol 25.3: A solution of **25.2** (0.18 g, 0.178 mmol) in ethyl acetate (5 mL)) and isopropyl alcohol (5 mL) was treated with 20% Pd(OH)2/C (0.09g) and stirred under a hydrogen atmosphere (balloon) for 24 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford **25.3** in quantitative yield.

Diethyl phosphonate 25.4: To a solution of compound **25.3** (0.124 g, 0.15 mmol) in acetonitrile (8 mL) and DMF (1 mL) was added potassium tert-butoxide (0.15 mL, 1M/THF). The mixture was stirred for 10 min. to form a clear solution. Diethyl triflate **5.3** (0.045 g, 0.15 mmol) was added to the reaction mixture. After stirred for 0.5 h, the reaction mixture was diluted with ethyl acetate and washed with HCl (0.1N). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **25.4** (0.039 g, 55% (based on recovered starting material: 0.064 g, 52%).

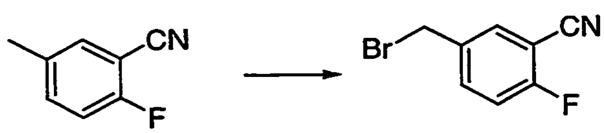
Bisindazole 25.6: A mixture of compound **25.4** (0.027 g), ethanol (1.5 mL), TFA (0.6 mL) and water (0.5 mL) was stirred at 60°C for 18 h. The mixture was concentrated under reduced pressure, and the residue was purified by HPLC to afford compound **25.6** as a TFA salt (0.014 g, 51%). ^{1}H NMR (CD3OD): δ 1.4 (t, J = 8 Hz, 6H), 2.9 (M, 4H), 3.2 (m, 2H), 3.58 (brs, 2H), 3.65 (m, 2H), 4.25 (m, 4H), 4.42 (d, J = 10 Hz, 2H), 4.85 (m, 2H), 6.75 (d, J = 9 Hz, 2H), 6.9 (m, 4H), 7.0 (d, J = 9 Hz, 2H), 7.4-7.6 (m, 6H), 8.1 (brs, 2H). 31P NMR (CD3OD): δ 20.8. MS: 769 (M+1).

Diethyl phosphonate 25.7: Compound **25.4** was converted into compound **25.7** in 76% yield according to the procedures described in Scheme 23 for the conversion of **23.3** into **23.5.**

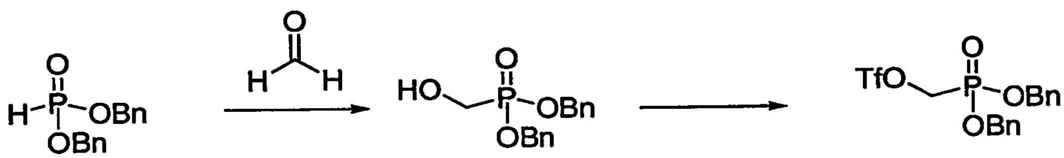
Bis indazole 25.8: Compound **25.7** (0.029 g) was treated in the same manner as compound **25.4** in the preparation of **25.6** to afford compound **25.8** as a TFA salt (0.0175 g, 59%). ^{1}H NMR (CD3OD): δ 1.4 (t, J = 8 Hz, 6H), 3.0 (M, 4H), 3.15 (d, J = 14 Hz, 1H), 3.25 (d, J = 14 Hz, 1H), 3.58 (brs, 2H), 3.65 (m, 2H), 4.25 (m, 4H), 4.42 (d, J = 10 Hz, 2H), 4.85 (m, 2H), 6.9 (d, J = 9 Hz, 2H), 7.0 (d, J = 9 Hz, 2H), 7.1 (d, J = 7 Hz, 2H), 7.2-7.6 (m, 9H), 8.1 (brs, 2H). ^{31}P NMR (CD3OD): δ 20.8. MS: 753 (M+1).

Preparation of Alkylating and Phosphonate Reagents

Scheme 50

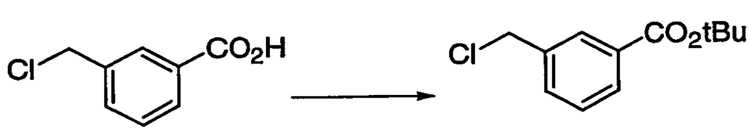


50.1 3.9

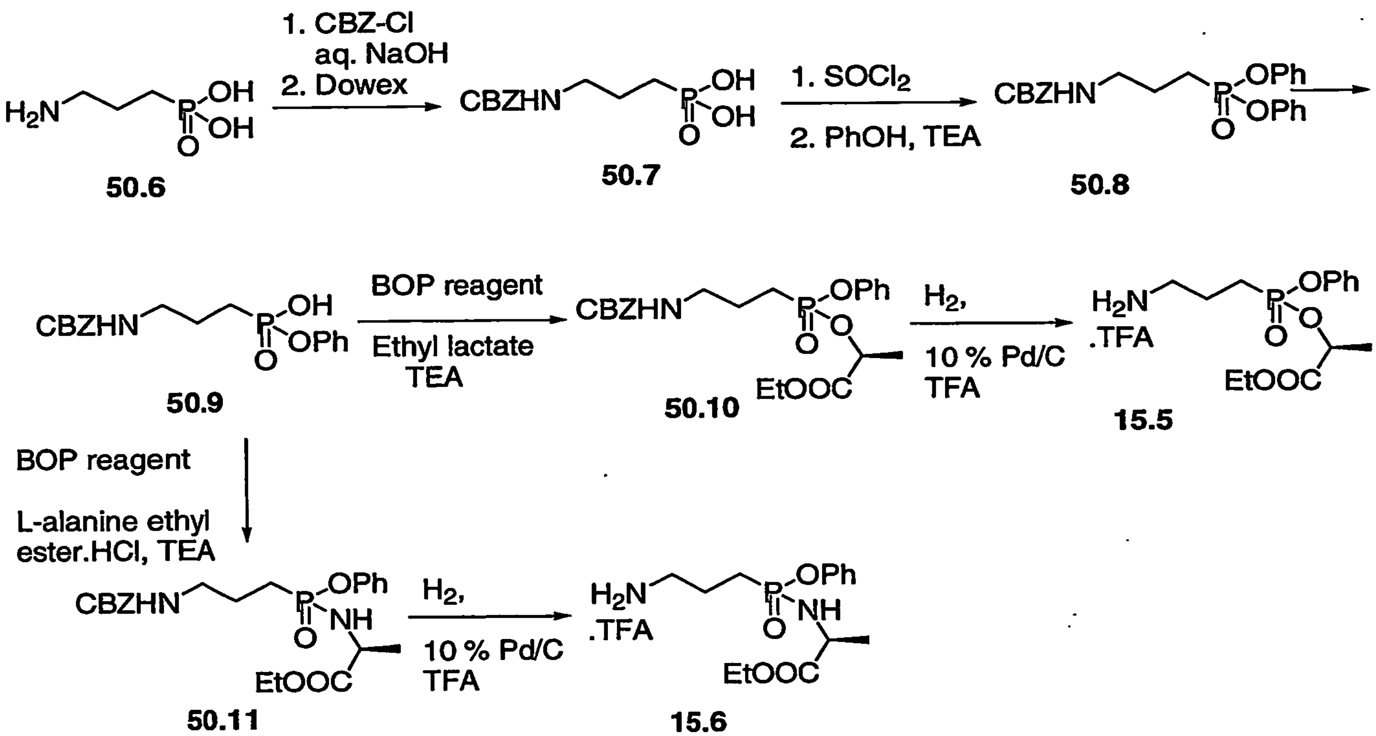


50.2 50.3 3.11

50.4 6.9



50.5 14.5



3-cyano-4-fluoro-benzylbromide 3.9: The commercially available 2—fluoro-4-methylbenzonitrile **50.1** (10 g, 74 mmol) was dissolved in carbon tetrachloride (50 mL) and then treated with NBS (16 g, 90 mmol) followed by AIBN (0.6 g, 3.7 mmol). The mixture was stirred at 85°C for 30 min and then allowed to cool to room temperature. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified by silica gel eluting with 5-20% ethyl acetate in hexanes to give **3.9** (8.8 g, 56%).

4-benzyloxy benzyl chloride 3.10 is purchased from Aldrich

Dibenzyl triflate 3.11: To a solution of dibenzyl phosphite **50.2** (100 g, 381 mmol) and formaldehyde (37% in water, 65 mL, 860 mmol) in THF (200 mL) was added TEA (5 mL, 36 mmol). The resulted mixture was stirred for 1 h, and then concentrated under reduced pressure. The residue was dissolved in methylene chloride and hexane (1:1, 300 mL), dried over sodium sulfate, filtered through a pad of silica gel (600 g) and eluted with ethyl acetate and hexane (1:1). The filtrate was concentrated under reduced pressure. The residue **50.3** (95 g) was dissolved in methylene chloride (800 mL), cooled to –78°C and then charged with pyridine (53 mL, 650 mmol). To this cooled solution was slowly added trifluoromethanesulfonic anhydride (120 g, 423 mmol). The resulted reaction mixture was

stirred and gradually warmed up to –15°C over 1.5 h period of time. The reaction mixture was cooled down to about – 50°C, diluted with hexane-ethyl acetate (2:1, 500 mL) and quenched with aqueous phosphoric acid (1M, 100 mL) at –10°C to 0°C. The mixture diluted with hexane-ethyl acetate (2:1, 1000 mL). The organic phase was washed with water, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford dibenzyl triflate **3.11** (66 g, 41%) as a colorless oil.

Diethyl triflate 5.3 is prepared as described in Tet Lett. 1986, 27, p1477-1480

3-Benzyloxybenzylbromide 6.9: To a solution of triphenyl phosphine (15.7 g, 60 mmol) in THF (150 mL) was added a solution of carbon tetrabromide (20 g, 60 mmol) in THF (50 mL). A precipitation was formed and stirred for 10 min. A solution of 3-benzyloxybenzyl alcohol **50.4** (10 g, 46.7 mmol) was added. After stirred for 1.5 h, the reaction mixture was filtered and concentrated under reduced pressure. The majority of triphenyl phosphine oxide was removed by precipitation from ethyl acetate-hexane. The crude product was purified by chromatography on silica gel and precipitation from hexane to give the desired product 3-Benzyloxybenzylbromide **6.9** (10 g, 77%) as a white solid.

t-Butyl-3-chloromethyl benzoate 14.5: A benzene solution (15 ml) of 3-chloromethylbenzoic acid **50.5** (1 g, 5.8 mmol) was heated at reflux, followed by the slow addition of N,N-dimethylforamide-di-t-butylacetal (5 m). The resulting solution was refluxed for 4 h, concentrated under reduced pressure and purified by silica gel column to afford **14.5** (0.8 g, 60 %).

Aminopropyl-diethylphosphonate 14.6 is purchased from Acros

Aminoethyl-diethylphosphonate oxalate 14.7 is purchased from Acros

Aminopropyl-phenol-ethyl lactate phosphonate 15.5

N-CBZ-aminopropyl diphenylphosphonate 50.8: An aqueous sodium hydroxide solution (50 mL of 1 N solution, 50 mmol) of 3-aminopropyl phosphonic acid **50.6** (3 g, 1.5 mmol)

was reacted with CBZ-Cl (4.1 g, 24 mmol) at room temperature overnight. The reaction mixture was washed with methylene chloride, acidified with Dowex 50wx8-200. The resin was filtered off. The filtrate was concentrated to dryness. The crude N-CBZ-aminopropyl phosphonic acid **50.7** (5.8 mmol) was suspended in CH_3CN (40 mL), and reacted with thionyl chloride (5.2 g, 44 mmol) at reflux for 4 hr, concentrated, and azeotroped with CH_3CN twice. The reaction mixture was redissolved in methylene chloride (20 mL), followed by the addition of phenol (3.2 g, 23 mmol), was cooled to 0°C. To this 0°C cold solution was added TEA (2.3 g, 23 mmol), and stirred at room temperature overnight. The reaction mixture was concentrated and purified on silica gel column chromatograph to afford **50.8** (1.5 g, 62 %).

Monophenol derivative 50.9: A CH_3CN solution (5 mL) of **50.8** (0.8 g, 1.88 mmol) was cooled to 0°C , and treated with 1N NaOH aqueous solution (4 mL, 4 mmol) for 2 h. The reaction was diluted with water, extracted with ethyl acetate, acidified with Dowex 50wx8-200. The aqueous solution was concentrated to dryness to afford **50.9** (0.56 g, 86%).

Monolactate derivative 50.10: A DMF solution (1 mL) of crude **50.9** (0.17 g, 0.48 mmol), BOP reagent (0.43 g, 0.97 mmol), ethyl lactate (0.12 g, 1 mmol), and DIPEA (0.31 g, 2.4 mmol) was reacted for 4 hr at room temperature. The reaction mixture was partitioned between methylene chloride and 5 % citric acid aqueous solution. The organic solution was separated, concentrated, and purified on preparative TLC to give **50.10** (0.14 g, 66%).

3-Aminopropyl lactate phosphonate 15.5: An ethyl acetate/ethanol solution (10 mL/2 mL) of **50.10** (0.14 g, 0.31 mmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (40 mg) for 3 hr. The catalyst was filtered off. The filtrate was concentrated to dryness to afford **15.5** (0.14 g, quantitative). NMR ($CDCl_3$): δ 8.0-8.2 (b, 3H), 7.1-7.4 (m, 5H), 4.9-5.0 (m, 1H), 4.15-4.3 (m, 2H), 3.1-3.35 (m, 2H), 2.1-2.4 (m, 4H), 1.4 (d, 3H), 1.3 (t, 3H).

Aminopropyl-phenol-ethyl alanine phosphonate 15.6: Compound **15.6** (80 mg) was prepared from the reaction of **50.9** (160 mg, 0.45 mmol) and L-alanine ethyl ester hydrochloride salt (0.11g, 0.68 mmol) in the presence of DIPEA and BOP reagent to give **50.11**, followed by the hydrogenation in the presence of 10% Pd/C and TFA to yield **15.6.** NMR ($CDCl_3$ + ~10 % CD_3OD): δ 8.0-8.2 (b), 7.25-7.35 (t, 2H), 7.1-7.2 (m, 3H), 4.0-4.15

(m, 2H), 3.8-4.0 (m, 1H), 3.0-3.1 (m, 2H), 1.15-1.25 (m, 6H). P NMR ($CDCl_3$ + ~10 % CD_3OD): 32.1 & 32.4 ppm.

Aminopropyl dibenzyl phosphonate 15.7 :

N-BOC-3-aminopropyl phosphonic acid 50.13: A THF-1N aqueous solution (16 mL-16 mL) of 3-aminopropyl phosphonic acid **50.12** (1 g, 7.2 mmol) was reacted with $(BOC)_2O$ (1.7 g, 7.9 mmol) overnight at room temperature. The reaction mixture was concentrated, and partitioned between methylene chloride and water. The aqueous solution was acidified with Dowex 50wx8-200. The resin was filtered off. The filtrate was concentrated to give **50.13** (2.2 g, 92 %).

N-BOC-3-aminopropyl dibenzyl phosphonate 50.14: A CH_3CN solution (10 mL) of **50.13** (0.15 g, 0.63 mmol), cesium carbonate (0.61 g, 1.88 mmol), and benzyl bromide (0.24 g, 1.57 mmol) was heated at reflux overnight. The reaction mixture was cooled to room temperature, and diluted with methylene chloride. The white solid was filtered off, washed thoroughly with methylene chloride. The organic phase was concentrated, and purified on preparative TLC to give **50.14** (0.18 g, 70%). MS: 442 (M + Na).

Aminopropyl dibenzyl phosphonate 15.7: A methylene chloride solution (1.6 mL) of **50.14** (0.18 g) was treated with TFA (0.4 mL) for 1 hr. The reaction mixture was concentrated to dryness, and azeotroped with CH_3CN twice to afford **15.7** (0.2 g, as TFA salt). NMR ($CDCl_3$): δ 8.6 (b, 2H), 7.9 (b, 2H), 7.2-7.4 (m, 10H), 4.71-5.0 (2 abq, 4H), 3.0 (b, 2H), 1.8-2 (m, 4H). 31P NMR ($CDCl_3$): 32.0 ppm. F NMR ($CDCl_3$): -76.5 ppm.

Aminomethyl diethylphosphonate 22.8 is purchased from Acros

Bromomethyl, tetrahydropyran indazole 25.9 is prepared according to J. Org. Chem. 1997, 62, p5627

<u>Activity of the CCPPI Compounds</u>

The enzyme inhibitory potency (Ki), antiviral activity (EC50), and cytotoxicity (CC50) of the tested compounds were measured and demonstrated.

Biological assays used for the characterization of PI prodrugs

HIV-1 Protease Enzyme Assay (*Ki*)

The assay is based on the fluorimetric detection of synthetic hexapeptide substrate cleavage by HIV-1 protease in a defined reaction buffer as initially described by M.V.Toth and G.R.Marshall, Int. J. Peptide Protein Res. 36, 544 (1990)

Substrate: (2-aminobenzoyl)Thr-Ile-Nle-(p-nitro)Phe-Gln-Arg
Substrate supplied by Bachem California, Inc. (Torrance, CA; Cat. no. H-2992)

Enzyme: recombinant HIV-1 protease expressed in E.Coli
Enzyme supplied by Bachem California, Inc. (Torrance, CA; Cat. no. H-9040)

Reaction buffer:	100 mM ammonium acetate, pH 5.3
	1 M sodium chloride
	1 mM ethylendiaminetetraacetic acid
	1 mM dithiothreitol
	10% dimethylsulfoxide

Assay protocol for the determination of inhibition constant Ki:

1. Prepare series of solutions containing identical amount of the enzyme (1 to 2.5 nM) and a tested inhibitor at different concentrations in the reaction buffer
2. Transfer the solutions (190 uL each) into a white 96-well plate
3. Preincubate for 15 min at 37°C
4. Solubilize the substrate in 100% dimethylsulfoxide at a concentration of 800 μM. Start the reaction by adding 10 μL of 800 μM substrate into each well (final substrate concentration of 40 μM)
5. Measure the real-time reaction kinetics at 37°C by using Gemini 96-well plate fluorimeter (Molecular Devices, Sunnyvale, CA) at λ(Ex) = 330 nm and λ(Em) = 420 nm
6. Determine initial velocities of the reactions with different inhibitor concentrations and calculate Ki (in picomolar concentration units) value by using EnzFitter program

(Biosoft, Cambridge, U.K.) according to an algorithm for tight-binding competitive inhibition described by Ermolieff J., Lin X., and Tang J., Biochemistry 36, 12364 (1997)

Anti-HIV-1 Cell Culture Assay *(EC_{50})*

The assay is based on quantification of the HIV-1-associated cytopathic effect by a colorimetric detection of the viability of virus-infected cells in the presence or absence of tested inhibitors. The HIV-1-induced cell death is determined using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) which is converted only by intact cells into a product with specific absorption characteristics as described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR, J. Natl. Cancer Inst. 81, 577 (1989).

Assay protocol for determination of EC_{50}:

1. Maintain MT2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
2. Infect the cells with the wild-type HIV-1 strain IIIB (Advanced Biotechnologies, Columbia, MD) for 3 hours at 37°C using the virus inoculum corresponding to a multiplicity of infection equal to 0.01.
3. Prepare a set of solutions containing various concentrations of the tested inhibitor by making 5-fold serial dilutions in 96-well plate (100 µL/well). Distribute the infected cells into the 96-well plate (20,000 cells in 100 µL/well). Include samples with untreated infected and untreated mock-infected control cells.
4. Incubate the cells for 5 days at 37°C.
5. Prepare XTT solution (6 mL per assay plate) at a concentration of 2mg/mL in a phosphate-buffered saline pH 7.4. Heat the solution in water-bath for 5 min at 55°C. Add 50 µL of N-methylphenazonium methasulfate (5 µg/mL) per 6 mL of XTT solution.
6. Remove 100 µL media from each well on the assay plate.
7. Add 100 µL of the XTT substrate solution per well and incubate at 37°C for 45 to 60 min in a CO_2 incubator.
8. Add 20 µL of 2% Triton X-100 per well to inactivate the virus.
9. Read the absorbance at 450 nm with subtracting off the background absorbance at 650 nm.

10. Plot the percentage absorbance relative to untreated control and estimate the EC_{50} value as drug concentration resulting in a 50% protection of the infected cells.

Cytotoxicity Cell Culture Assay *(CC_{50})*:

The assay is based on the evaluation of cytotoxic effect of tested compounds using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) as described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR, J. Natl. Cancer Inst. 81, 577 (1989).

Assay protocol for determination of CC_{50}:

1. Maintain MT-2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
2. Prepare a set of solutions containing various concentrations of the tested inhibitor by making 5-fold serial dilutions in 96-well plate (100 µL /well). Distribute cells into the 96-well plate (20,000 cells in 100 µL/well). Include samples with untreated cells as a control.
3. Incubate the cells for 5 days at 37°C.
4. Prepare XTT solution (6 mL per assay plate) in dark at a concentration of 2mg/mL in a phosphate-buffered saline pH 7.4. Heat the solution in a water-bath at 55°C for 5 min. Add 50 µL of N-methylphenazonium methasulfate (5 µg/mL) per 6 mL of XTT solution.
5. Remove 100 µL media from each well on the assay plate and add 100 µL of the XTT substrate solution per well. Incubate at 37°C for 45 to 60 min in a CO_2 incubator.
6. Add 20 µL of 2% Triton X-100 per well to stop the metabolic conversion of XTT.
7. Read the absorbance at 450 nm with subtracting off the background at 650 nm.
8. Plot the percentage absorbance relative to untreated control and estimate the CC50 value as drug concentration resulting in a 50% inhibition of the cell growth. Consider the absorbance being directly proportional to the cell growth.

Resistance Evaluation *(I50V and I84V/L90M fold change)*

The assay is based on the determination of a difference in the susceptibility to a particular HIV protease inhibitor between the wild-type HIV-1 strain and a mutant HIV-1 strain

containing specific drug resistance-associated mutation(s) in the viral protease gene. The absolute susceptibility of each virus (EC_{50}) to a particular tested compound is measured by using the XTT-based cytopathic assay as described above. The degree of resistance to a tested compound is calculated as fold difference in EC_{50} between the wild type and a specific mutant virus. This represents a standard approach for HIV drug resistance evaluation as documented in various publications (e.g. Maguire et al., Antimicrob. Agents Chemother. 46: 731, 2002; Gong et al., Antimicrob. Agents Chemother. 44: 2319, 2000; Vandamme and De Clercq, in Antiviral Therapy (Ed. E. De Clercq), pp. 243, ASM Press, Washington, DC, 2001).

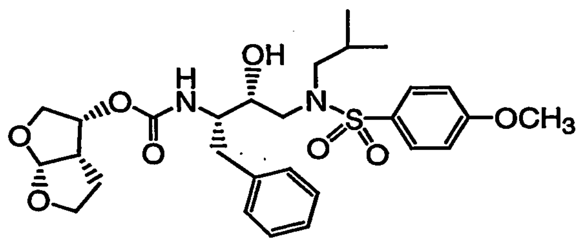
<u>HIV-1 strains used for the resistance evaluation:</u>

Two strains of mutant viruses containing I50V mutation in the protease gene have been used in the resistance assays: one with M46I/I47V/I50V mutations (designated I50V #1) and the other with L10I/M46I/I50V (designated I50V #2) mutations in the viral protease gene. A third virus with I84V/L90M mutations was also employed in the resistance assays. Mutants I50V #1 and I84V/L90M were constructed by a homologous recombination between three overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with the protease and reverse transcriptase genes deleted, 2. DNA fragment generated by PCR amplification containing reverse transcriptase gene from HXB2D strain (wild-type), 3. DNA fragment of mutated viral protease gene that has been generated by PCR amplification. An approach similar to that described by Shi and Mellors in Antimicrob. Agents Chemother. 41: 2781-85, 1997 was used for the construction of mutant viruses from the generated DNA fragments. Mixture of DNA fragments was delivered into Sup-T1 cells by using a standard electroporation technique. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics until the recombinant virus emerged (usually 10 to 15 days following the electroporation). Cell culture supernatant containing the recombinant virus was harvested and stored in aliquots. After verification of protease gene sequence and determination of the infectious virus titer, the viral stock was used for drug resistance studies. Mutant I50V #2 is an amprenavir-resistant HIV-1 strain selected *in vitro* from the wild-type IIIB strain in the presence of increasing concentration of amprenavir over a period of > 9 months using an approach similar to that described by Partaledis et al., J. Virol. 69: 5228-5235, 1995. Virus capable of growing in the presence of 5

μM amprenavir was harvested from the supernatant of infected cells and used for resistance assays following the titration and protease gene sequencing.

Example 37: Activity of the Tested Compounds

The enzyme inhibitory potency (Ki), antiviral activity (EC50), and cytotoxicity (CC50) of the tested compounds are summarized in Table 1.



94-003

Table 1: Enzyme inhibition activity (Ki), antiviral cell culture activity (EC50), and cytotoxicity (CC50) of the tested compounds.

Substitution of (P1)phenyl	Compound	Phosphonate substitution	HIV-1 protease inhibition Ki [pM]	Anti-HIV-1 Cell Culture Activity EC50 [nM]	Cytotoxicity CC50 [μM]
none	Amprenavir	none	45.6 ± 18.2	16 ± 2.2	
none	94-003	none	1.46 ± 0.58	1.4 ± 0.3	
phosphonyl	27	diacid	11.8 ± 6.0	> 100,000	> 100
	28	diethyl	1.2 ± 0.8	5.0 ± 2.8	70
phosphonyl methoxy	11	diacid	2.1 ± 0.2	4,800 ± 1,800	> 100
	13	diethyl	2.6 ± 1.5	3.0 ± 0	50
	14	dibenzyl	12.7 ± 1.9	2.3 ± 0.4	35
	16c	bis(Ala-ethylester)	15.4 ± 0.85	105 ± 43	60
	16d	bis(Ala-butylester)	18.75 ± 3.04	6.0 ± 1.4	
	16e	bis(ABA-ethylester)	8.8 ± 1.7	12.5 ± 3.5	
	16f	bis(ABA-butylester)	3.5 ± 1.4	4.8 ± 1.8	
	16a	bis(Gly-ethylester)	29 ± 8.2	330 ± 230	
	16b	bis(Gly-butylester)	4.9 ± 1.8	17.5 ± 10.5	
	16g	bis(Leu-ethylester)	29 ± 9	6.8 ± 0.4	
	16h	bis(Leu-butylester)	31.7 ± 19.3	120 ± 42	
	16i	bis(Phe-ethylester)		17 ± 12	
	16j	bis(Phe-butylester)		35 ± 7	
	15	bis(POC)	36	825 ± 106	
	11	Monoethyl, monoacid	0.45 ± 0.15	700 ± 0	

<u>Cross-Resistance Profile Assay</u>

The assay is based on the determination of a difference in the susceptibility to a particular HIV protease inhibitor between the wild-type HIV-1 strain and a recombinant HIV-1 strain expressing specific drug resistance-associated mutation(s) in the viral protease gene. The absolute susceptibility of each virus to a particular tested compound is measured by using the XTT-based cytopathic assay as described in Example B. The degree of resistance to a tested compound is calculated as fold difference in EC50 between the wild type and a specific mutant virus.

Recombinant HIV-1 strains with resistance mutations in the protease gene:

One mutant virus (82T/84V) was obtained from NIH AIDS Research and Reference Reagent Program (Rockville, MD). Majority of the mutant HIV-1 strains were constructed by a homologous recombination between three overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with the protease and reverse transcriptase genes deleted, 2. DNA fragment generated by PCR amplification containing reverse transcriptase gene from HXB2D strain (wild-type), 3. DNA fragment generated by RT-PCR amplification from patients plasma samples containing viral protease gene with specific mutations selected during antiretroviral therapy with various protease inhibitors. Additional mutant HIV-1 strains were constructed by a modified procedure relying on a homologous recombination of only two overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with only the protease gene deleted, and 2. DNA fragment generated by RT-PCR amplification from patients plasma samples containing viral protease gene with specific mutations. In both cases, mixture of DNA fragments was delivered into Sup-T1 cells by using a standard electroporation technique. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics until the recombinant virus emerged (usually 10 to 15 days following the electroporation). Cell culture supernatant containing the recombinant virus was harvested and stored in aliquots. After determination of the virus titer the virus stock was used for drug resistance studies.

Example 39: Cross-Resistance Profile of the Tested Compounds

Cross-resistance profile of currently used HIV-1 protease inhibitors was compared with that of the newly invented compounds (Table 2).

Table 2. Cross-resistance profile of HIV-1 protease inhibitors

Compound	EC 50 [nM] WT HIV -1	Fold Change in EC_{50} Relative to WT HIV-1											Total No. of Resistant Viruses [b]
		8K[a] 46I **90M**	46I **84A**	10I **48V** 54V **82A**	46I 47V **50V**	10R 46I **82T** **84V**	**30N** 50S **82I** 88D	54V 71V **82S**	10F 46I 71V **82T** **90M**	10I **48V** 71V **82A** **90M**	**48V** 54V 71V **82S**	10I **84V** 71V 73S **90M**	
Amprenavir	20	1.25	14	2	38	4	0.8	4	13	2.5	2	10	4
Nelfinavir	14	13	11	11.5	2	3	43	12	33	27	12	65	9
Indinavir	15	4	10	15	nd	7	1	10	13	28	23	43	8
Ritonavir	15	34	18	20	13	47	2	20	32	22	>50	42	10
Saquinavir	4	1	2.5	11	1	2.5	1	3	2.5	12	45	40	4
Lopinavir	8	nd	9	nd	19	11	nd	nd	7.5	4.5	60	11	6
Tipranavir	80	nd	1	0.4	0.5	5	0.5	3.5	3	0.3	2	nd	1
94-003	0.5	nd	8	0.5	29	nd	0.4	3.5	nd	nd	nd	8	3
GS 16503	16	1.2	1	0.4	3.3	1	0.6	0.9	1	0.4	0.5	2	0
GS 16571	22	1.8	1	0.3	0.8	0.6	0.7	0.6	0.8	0.2	0.2	0.9	0
GS 16587	15	1.5	1	0.5	2	1	1	0.9	1	0.4	0.4	1	0

[a] Resistance-associated mutations present in the viral protease. The highlighted changes represent primary resistance mutations.

[b] Resistance is considered as a 5-fold and higher change in the EC50 value of the mutant virus relative to the wild-type virus.

Example Section N

Plasma and PBMC Exposure Following Intravenous and Oral Administration of Prodrug to Beagle Dogs

The pharmacokinetics of a phosphonate prodrug GS77366 (P1-monoLac-iPr), its active metabolite (metabolite X, or GS77568), and GS8373 were studied in dogs following intravenous and oral administration of the prodrug.

Dose Administration and Sample Collection. The in-life phase of this study was conducted in accordance with the USDA Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and followed the standards for animal husbandry and care found in the Guide for the Care and Use of Laboratory Animals, 7th Edition, Revised 1996. All animal housing and study procedures involving live animals were carried out at a facility which had been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care - International (AAALAC).

Each animal in a group of 4 female beagle dogs was given a bolus dose of GS77366 (P1-monoLac-iPr) intravenously at 1 mg/kg in a formulation containing 40% PEG 300, 20% propylene glycol and 40% of 5% dextrose. Another group of 4 female beagle dogs was dosed with GS77366 via oral gavage at 20 mg/kg in a formulation containing 60% Vitamin-E TPGS, 30% PEG 400 and 10% propylene glycol.

Blood samples were collected pre-dose, and at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr post-dose. Plasma (0.5 to 1 mL) was prepared from each sample and kept at -70°C until analysis. Blood samples (8 mL) were also collected from each dog at 2, 8 and 24 hr post dose in Becton-Dickinson CPT vacutainer tubes. PBMCs were isolated from the blood by centrifugation for 15 minutes at 1500 to 1800 G. After centrifugation, the fraction containing PBMCs was transferred to a 15 mL conical centrifuge tube and the PBMCs were washed twice with phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}. The final wash of the cell pellet was kept at -70°C until analysis.

Measurement of the prodrug, metabolite X and GS8373 in plasma and PBMCs. For plasma sample analysis, the samples were processed by a solid phase extraction (SPE) procedure outlined below. Speedisk C18 solid phase extraction cartridges (1 mL, 20 mg, 10 μM, from

J.T. Baker) were conditioned with 200 µL of methanol followed by 200 µL of water. An aliquot of 200 µL of plasma sample was applied to each cartridge, followed by two washing steps each with 200 µL of deionized water. The compounds were eluted from the cartridges with a two-step process each with 125 µL of methanol. Each well was added 50 µL of water and mixed. An aliquot of 25 µL of the mixture was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography was HyPURITY® C18 (50 x 2.1 mm, 3.5 um) from Thermo-Hypersil. Mobile phase A contained 10% acetonitrile in 10 mM ammonium formate, pH 3.0. Mobile phase B contained 90% acetonitrile in 10 mM ammonium formate, pH 4.6. The chromatography was carried out at a flow rate of 250 µL/min under an isocratic condition of 40% mobile phase A and 60% mobile phase B. Selected reaction monitoring (SRM) were used to measure GS77366, GS8373 and Metabolite X with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 1 nM for GS77366, GS8373 and GS77568 (Metabolite X) in plasma.
For PBMC sample analysis, phosphate buffered saline (PBS) was added to each PBMC pellet to bring the total sample volume to 500 µL in each sample. An aliquot of 150 µL from each PBMC sample was mixed with an equal volume of methanol, followed by the addition of 700 µL of 1% formic acid in water. The resulting mixture was applied to a Speedisk C18 solid phase extraction cartridge (1 mL, 20 mg, 10 um, from J.T. Baker) which had been conditioned as described above. The compounds were eluted with methanol after washing the cartridge 3 times with 10% methanol. The solvent was evaporated under a stream of N_2, and the sample was reconstituted in 150 µL of 30% methanol. An aliquot of 75 µL of the solution was injected for LC/MS/MS analysis. The limit of quantitation was 0.1 ng/mL in the PBMC suspension.

Pharmacokinetic Calculations. The pharmacokinetic parameters were calculated using WinNonlin. Noncompartmental analysis was used for all pharmacokinetic calculation. The intracellular concentrations in PBMCs were calculated from the measured concentrations in PBMC suspension on the basis of a reported volume of 0.2 picoliter/cell (B.L. Robins, R.V. Srinivas, C.Kim, N.Bischofberger, and A.Fridland, (1998) Antimicrob. Agents Chemother. 42, 612).

Plasma and PBMC Concentration-time Profiles.

The concentration-time profiles of GS77366, GS77568 and GS8373 in plasma and PBMCs following intravenous dosing of GS77366 were compared at 1 mg/kg in dogs. The data demonstrate that the prodrug can effectively deliver the active components (metabolite X and GS8373) into cells that are primarily responsible for HIV replication, and that the active components in these cells had much longer half-life than in plasma.

The pharmacokinetic properties of GS77568 in PBMCs following oral administration of GS77366 in dogs are compared with that of nelfinavir and amprenavir, two marketed HIV protease inhibitors (Table 3). These data show that the active component (GS77568) from the phosphonate prodrug had sustained levels in PBMCs compared to nelfinavir and amprenavir.

Table 3. Comparison of GS77568 with nelfinavir and amprenavir in PBMCs following oral administration in beagle dogs.

Compound	Dose	$t_{1/2}$ (hr)	$AUC_{(2\text{-}24\ hr)}$
Nelfinavir	17.5 mg/kg	3.0 hr	33,000 nM·hr
Amprenavir	20 mg/kg	1.7 hr	102,000 nM·hr
GS77568	20 mg/kg of GS77366	> 20 hr	42,200 nM·hr

Example Section O

Intracellular Metabolism/In Vitro Stability

1. Uptake and Persistence in MT2 cells, quiescent and stimulated PBMC

The protease inhibitor (PI) phosphonate prodrugs undergo rapid cell uptake and metabolism to produce acid metabolites including the parent phosphonic acid. Due to the presence of charges, the acid metabolites are significantly more persistent in the cells than non-charged PI's. In order to estimate the relative intracellular levels of the different PI prodrugs, three compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate, monoamidate phenoxy phosphonate and monolactate phenoxy phosphonate (Figure 1) were incubated at 10 µM for 1 hr with MT-2 cells, stimulated and quiescent peripheral blood mononuclear cells (PBMC) (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 24 hr (chase phase). At specific time points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. Typically, the cell lysates were centrifuged and 100 uL of the supernatant were mixed with 200 µL of 7.5 uM amprenavir (Internal Standard) in 80% acetonitrile/20% water and injected into an HPLC system (70 µL).

HPLC Conditions:

Analytical Column: Prodigy ODS-3, 75 x 4.6, 3u + C18 guard at 40°C

Gradient:

Mobile Phase A: 20 mM ammonium acetate in 10% ACN/90% H_2O

Mobile Phase B: 20 mM ammonium acetate in 70% ACN/30% H_2O

30-100%B in 4 min, 100%B for 2 min, 30%B for 2 min at 2.5 mL/min.

Run Time: 8 min

UV Detection at 245 nm

Concentrations of Intracellular metabolites were calculated based on cell volume 0.2 µL/mLn cells for PBMC and 0.338 µL / mLn (0.676 uL / mL) for MT-2 cells.

Chemical Structures of Selected Protease Inhibitor Phosphonate Prodrugs and Intracellular Metabolites:

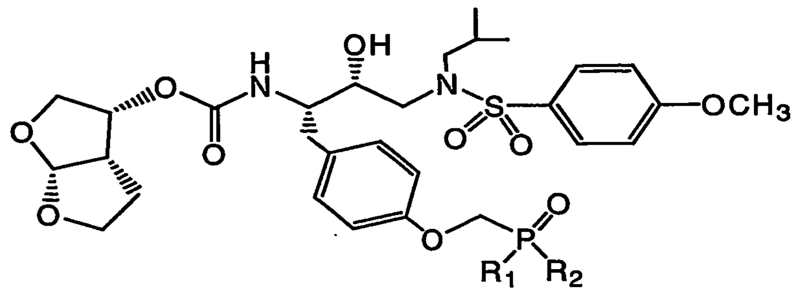


Table 4:

GS No.	R1	R2	EC_{50} (nM)
8373	OH	OH	4,800±1,800
16503	$HNCH(CH_3)COOBu$	$HNCH(CH_3)COOBu$	6.0±1.4
16571	OPh	$HNCH(CH_3)COOEt$	15±5
17394	OPh	$OCH(CH_3)COOEt$	20±7
16576	OPh	$HNCH(CH_2CH_3)COOEt$	12.6±4.8
Met X	OH	$HNCH(CH_3)COOH$	>10,000
Met LX	OH	$OCH(CH_3)COOEt$	1750±354

A significant uptake and conversion of all 3 compounds in all cell types was observed (Table 4). The uptake in the quiescent PBMC was 2-3-fold greater than in the stimulated cells. GS-16503 and GS-16571 were metabolized to Metabolite X and GS-8373. GS-17394 metabolized to the Metabolite LX. Apparent intracellular half-lives were similar for all metabolites in all cell types (7-12 hr). A persistence of Total Acid Metabolites of Protease Inhibitor Prodrugs in Stimulated (A), Quiescent PBMC (B) and MT-2 Cells (C) (1 hr, 10 uM Pulse, 24 hr Chase) was observed.

2. Uptake and Persistence in Stimulated and Quiescent T-cells

Since HIV mainly targets T-lymphocytes, it is important to establish the uptake, metabolism and persistence of the metabolites in the human T-cells. In order to estimate the relative intracellular levels of the different PI prodrugs, GS-16503, 16571 and 17394 were incubated at 10 μM for 1 hr with quiescent and stimulated T-cells (pulse phase). The prodrugs were compared with a non-prodrug PI, nelfinavir. After incubation, the cells were washed, resuspended in the cell culture media and incubated for 4 hr (chase phase). At specific time

points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. The sample preparation and analysis were similar to the ones described for MT-2 cells, quiescent and stimulated PBMC.

Table 5 demonstrate the levels of total acid metabolites and corresponding prodrugs in T-cells following pulse/chase and continuous incubation. There was significant cell uptake/metabolism in T-lymphocytes. There was no apparent difference in uptake between stimulated and quiescent T-lymphocytes. There was significantly higher uptake of phosphonate PI's than nelfinavir. GS17394 demonstrates higher intracellular levels than GS16571 and GS16503. The degree of conversion to acid metabolites varied between different prodrugs. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571. The metabolites, generally, were an equal mixture of the mono-phosphonic acid metabolite and GS-8373 except for GS-17394, where Metabolite LX was stable, with no GS-8373 formed.

Table 5. Intracellular Levels of Metabolites and Intact Prodrug Following Continuous and 1 hr Pulse/4 hr Chase Incubation (10 μM/0.7 mLn cells/1 mL) of 10 μM PI Prodrugs and Nelfinavir with Quiescent and Stimulated T-cell

		Continuous Incubation				1 hr Pulse /4 hr Chase			
		Quiescent T-cells		Stimulated T-cells		Quiescent T-cells		Stimulated T-cells	
Compound	Time (h)	Acid Met (μM)	Prodrug (μM)	Acid Met (μM)	Prodrug (μM)	Acid Met (μM)	Prodrug (μM)	Acid Met (μM)	Prodrug (μM)
16503	0	1180	42	2278	0	2989	40	1323	139
	2	3170	88	1083	116	1867	4	1137	31
	4	5262	0	3198	31	1054	119	1008	0
16571	0	388	1392	187	1417	1042	181	858	218
	2	947	841	1895	807	1170	82	1006	35
	4	3518	464	6147	474	1176	37	616	25
17394	0	948	1155	186	1194	4480	14	2818	10
	2	7231	413	3748	471	2898	33	1083	51
	4	10153	167	3867	228	1548	39	943	104
Nelfinavir	0		101		86		886		1239
	2		856		846		725		770
	4		992		1526		171		544

3. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 μM.

To were similar to the determine if the cell uptake/metabolism is concentration dependent, selected PI's were incubated with the 1 mL of MT-2 cell suspension (2.74 mLn cells/mL) for 1 hr at 37°C at 3 different concentrations: 10, 5 and 1 μM. Following incubation, cells were washed twice with the cell culture medium, lysed and assayed using HPLC with UV detection. The sample preparation and analysis ones described for MT-2 cells, quiescent and stimulated PBMC. Intracellular concentrations were calculated based on cell count, a published single cell volume of 0.338 pl for MT-2 cells, and concentrations of analytes in cell lysates. Data are shown in Table 6.

Uptake of all three selected PI's in MT-2 cells appears to be concentration-independent in the 1-10 μM range. Metabolism (conversion to acid metabolites) appeared to be concentration-dependent for GS-16503 and GS-16577 (3-fold increase at 1 μM vs. 10 μM) but independent for GS-17394 (monolactate). Conversion from a respective metabolite X to GS-8373 was concentration-independent for both GS-16503 and GS-16577 (no conversion was observed for metabolite LX of GS-17394).

Table 6. Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 μM.

Compound	Extracellular Concentration, μM	Cell-Assosiated Prodrug and Metabolites Concentration, μM				% Conversion to acid metabolites
		Metabolite X	GS8373	Prodrug	Total	
GS-17394	10	1358	0	635	1993	68
	5	916	0	449	1365	67
	1	196	0	63	260	76
GS-16576	10	478	238	2519	3235	22
	5	250	148	621	1043	40
	1	65	36	61	168	64
GS-16503	10	120	86	1506	1712	12
	5	58	60	579	697	17
	1	12	18	74	104	29

* For GS16576, Metabolite X is mono-aminobutyric acid

4. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10 μM.

In order to estimate the relative intracellular levels of the different PI prodrugs under conditions simulating the in vivo environment, compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate (GS-16503), monoamidate phenoxy phosphonate (GS-16571) and monolactate phenoxy phosphonate(GS-17394) were incubated at 10 μM for 1 hr with intact human whole blood at 37°C. After incubation, PBMC were isolated, then lysed and the lysates were analyzed by HPLC with UV detection. The results of analysis are shown in Table 7. There was significant cell uptake/metabolism following incubation in whole blood. There was no apparent difference in uptake between GS-16503 and GS-16571. GS-17394 demonstrated significantly higher intracellular levels than GS-16571 and GS-16503.

The degree of conversion to acid metabolites varies between different prodrugs after 1 hr incubation. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571 (Table 7). The metabolites, generally, were an equimolar mixture of the mono-phosphonic acid metabolite and GS-8373 (parent acid) except for GS-17394, where Metabolite LX was stable with no GS-8373 formed.

Table 7. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10 μM (Mean ± SD, N=3).

GS#	Intracellular Prodrug and Metabolites Concentration, μM			Major Intracellular Metabolites
	Acid Metabolite	Prodrug, μM	Total, μM	
16503	279 ± 47	61 ± 40	340 ± 35	X , GS-8373
16571	319 ± 112	137 ± 62	432 ± 208	X, GS-8373
17394	629 ± 303	69 ± 85	698 ± 301	LX

* PBMC Intracellular Volume = 0.2 µL/mln

5. Distribution of PI Prodrugs in PBMC

In order to compare distribution and persistence of PI phosphonate prodrugs with those of non-prodrug PI's, GS-16503, GS-17394 and nelfinavir, were incubated at 10 µM for 1 hr with PBMC (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 20 more hr (chase phase). At specific time points, the cells were washed and lysed. The cell cytosol was separated from membranes by centrifugation at 9000 xg. Both cytosol and membranes were extracted with acetonitrile and analyzed by HPLC with UV detection.

Table 8 shows the levels of total acid metabolites and corresponding prodrugs in the cytosol and membranes before and after the 22 hr chase. Both prodrugs exhibited complete conversion to the acid metabolites (GS-8373 and X for GS-16503 and LX for GS-17394, respectively). The levels of the acid metabolites of the PI phosphonate prodrugs in the cytosol fraction were 2-3-fold greater than those in the membrane fraction after the 1 hr pulse and 10-fold greater after the 22 hr chase. Nelfinavir was present only in the membrane fractions. The uptake of GS-17394 was about 3-fold greater than that of GS-16503 and 30-fold greater than nelfinavir. The metabolites were an equimolar mixture of metabolite X and GS-8373 (parent acid) for GS-16503 and only metabolite LX for GS-17394.

Table 8. Uptake and Cell Distribution of Metabolites and Intact Prodrugs Following Continuous and 1 hr Pulse/22 hr Chase Incubation of 10 μM PI Prodrugs and Nelfinavir with Quiescent PBMC.

GS#	Cell Type	Fraction	Cell-Associated PI, pmol/mln cells			
			1 hr Pulse/ 0 hr Chase		1 hr Pulse/ 22 hr Chase	
			Acid Metabolites	Prodrug	Acid Metabolites	Prodrug
GS-16503	PBMC	Membrane	228	0	9	0
GS-16503	PBMC	Cytosol	390	0	130	0
GS-17394	PBMC	Membrane	335	0	26	0
GS-17394	PBMC	Cytosol	894	0	249	0
Nelfinavir	PBMC	Membrane		42		25
Nelfinavir	PBMC	Cytosol		0		0

Uptake and cell distribution of metabolites and intact prodrugs following 1 hr pulse/22 hr chase incubation of 10 μM PI prodrugs and Nelfinavir with quiescent PBMC were measured.

6. PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

The *in vitro* metabolism and stability of the PI phosphonate prodrugs were determined in PBMC extract, dog plasma and human serum (Table 9). Biological samples listed below (120 μL) were transferred into an 8-tube strip placed in the aluminum 37°C heating block/holder and incubated at 37°C for 5 min. Aliquots (2.5 μL) of solution containing 1 mM of test compounds in DMSO, were transferred to a clean 8-tube strip, placed in the aluminum 37°C heating block/holder. 60 μL aliquots of 80% acetonitrile/20% water containing 7.5 μM of amprenavir as an internal standard for HPLC analysis were placed into five 8-tube strips and kept on ice/refrigerated prior to use. An enzymatic reaction was started by adding 120 μL aliquots of a biological sample to the strip with the test compounds using a multichannel pipet. The strip was immediately vortex-mixed and the reaction mixture (20 μL) was sampled and transferred to the Internal Standard/ACN strip. The sample was considered the time-zero sample (actual time was 1-2 min). Then, at specific time points, the

reaction mixture (20 μL) was sampled and transferred to the corresponding IS/ACN strip. Typical sampling times were 6, 20, 60 and 120 min. When all time points were sampled, an 80 μL aliquot of water was added to each tube and strips were centrifuged for 30 min at 3000xG. The supernatants were analyzed with HPLC under the following conditions:

Column: Inertsil ODS-3, 75 x 4.6 mm, 3 μm at 40°C.
Mobile Phase A: 20 mM ammonium acetate in 10%ACN/90%water
Mobile Phase B 20 mM ammonium acetate in 70%ACN/30%water
Gradient: 20% B to 100% B in 4 min, 2 min 100% B, 2 min 20% B
Flow Rate: 2 mL/min
Detection: UV at 243 nm
Run Time: 8 min

The biological samples evaluated were as follows:

<u>PBMC cell extract</u> was prepared from fresh cells using a modified published procedure (A. Pompon, I. Lefebvre, J-L. Imbach, S. Kahn, and D. Farquhar, Antiviral Chemistry & Chemotherapy, 5, 91 - 98 (1994)). Briefly, the extract was prepared as following: The cells were separated from their culture medium by centrifugation (1000 g, 15 min, ambient temperature). The residue (about 100 μL, 3.5 x 10^8 cells) was resuspended in 4 mL of a buffer (0.010 M HEPES, pH 7.4, 50 mM potassium chloride, 5 mM magnesium chloride and 5 mM dl-dithiothreitol) and sonicated. The lysate was centrifuged (9000 g, 10 min, 4°C) to remove membranes. The upper layer (0.5 mg protein/mL) was stored at –70°C. The reaction mixture contained the cell extract at about 0.5 mg protein/mL.

<u>Human serum</u> (pooled normal human serum from George King Biomedical Systems, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

<u>Dog Plasma</u> (pooled normal dog plasma (EDTA) from Pel Freez, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

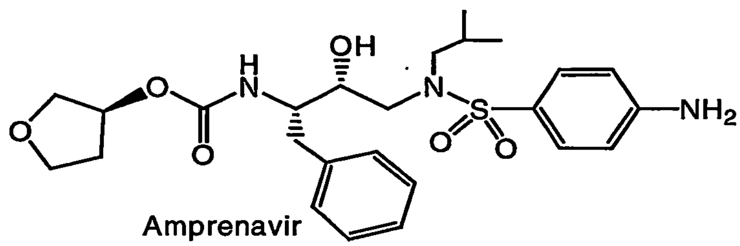
Table 9: PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

GS#	PBMC Extract[1] $T_{1/2}$, min	Dog Plasma $T_{1/2}$, min	Human Serum $T_{1/2}$, min	HIV EC_{50} (nM)
16503	2	368	>>400	6.0 ± 1.4
16571	49	126	110	15 ± 5
17394	15	144	49	20 ± 7

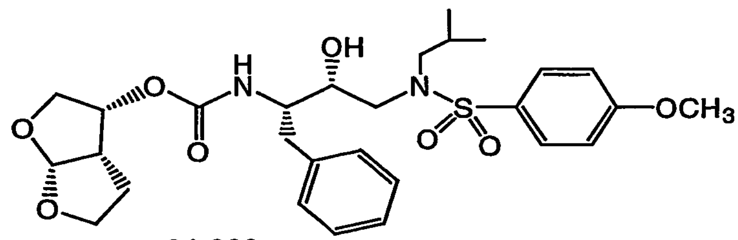
Example Section P

Table 10: Enzymatic and Cellular data

Formula II ALPPI activity



Amprenavir



94-003

Ki [pM]

≤ 10	+++
> 10 to ≤ 100	++
> 100 to ≤ 1,000	+
> 1,000	-

EC_{50} [nM]

≤ 50	+++
> 50 to ≤ 500	++
> 500 to ≤ 5,000	+
> 5,000	-

I50V and I84V/L90M fold change

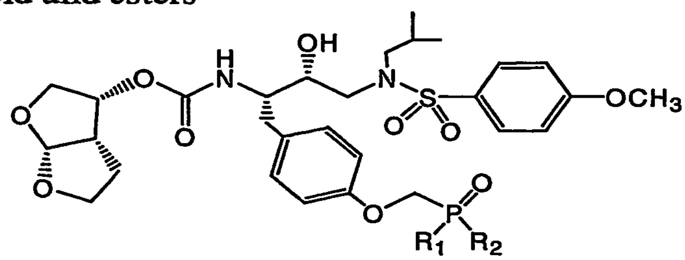
> 30	+++
> 10 to ≤ 30	++
> 3 to ≤ 10	+
≤ 3	-

CC_{50} [µM]

≤ 5	++
> 5 to ≤ 50	+
> 50	-

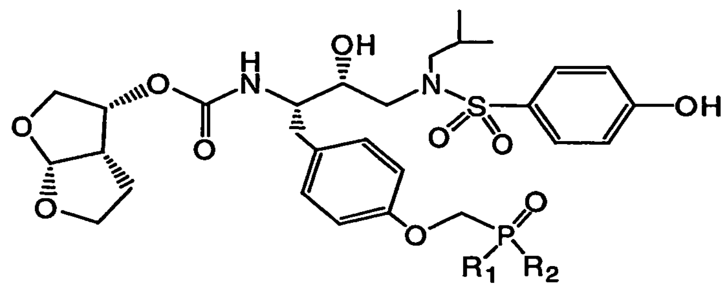
Compound	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I50V (#2) fold change	I84V/L90M fold change	CC_{50} (μM)
Saquinavir	++	+++	–	–	+++	
Nelfinavir	+	+++	–	+	+++	
Indinavir	+	+++	–	+	+++	
Ritonavir	++	+++	++	++	+++	
Lopinavir	++	+++	++	+++	++	
Amprenavir	+	+++	+++	+++	++	–
Atazanavir	++	+++	–	–	+++	
Tipranavir	++	++	–	–	+	
94-003	+++	+++	+++	+++	++	+
TMC114	+++	+++	++	++	–	

P1-Phosphonic acid and esters



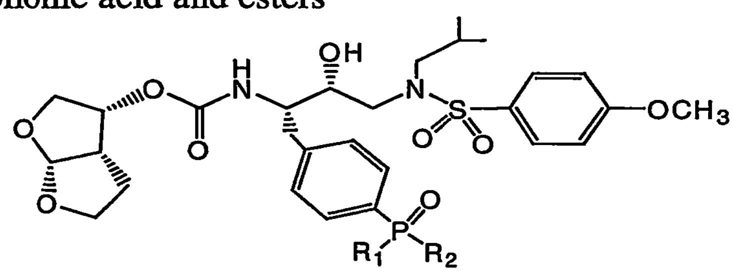
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} (μM)
OH	OH	+++	+	–	–	–
OMe	OMe	++	+++			
OEt	OEt	+++	+++	–	–	+
OCH_2CF_3	OCH_2CF_3	++	–			
OiPr	OiPr	++	+++	–	–	
OPh	OPh		+++			
OMe	OPh	++	+++			
OEt	OPh	+++	+++			
OBn	OBn	++	+++	–	–	+
OEt	OBn	++	+++			++
OPoc	OPoc		+			
OH	OEt		++			
OH	OPh	+++	–			
OH	OBn		+	–	–	

P1-Phosphonic acid and esters



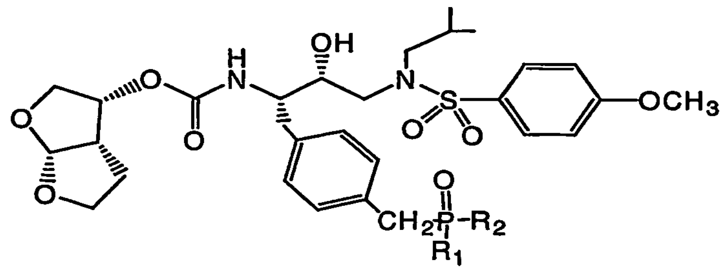
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} (μM)
OH	OH	+++	+			
Et	Et	+++	+++			

P1-Direct phosphonic acid and esters



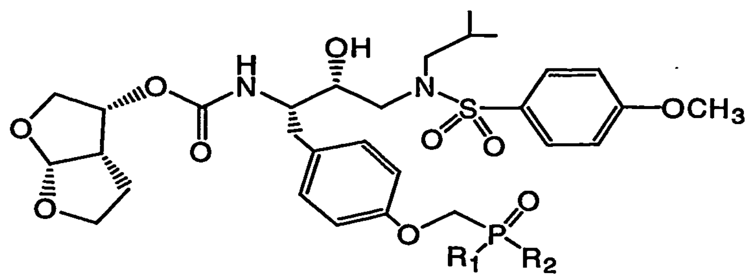
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
OH	OH	++	–			
OEt	OEt	+++	+++	+	–	

P1-CH_2-phosphonic acid and esters



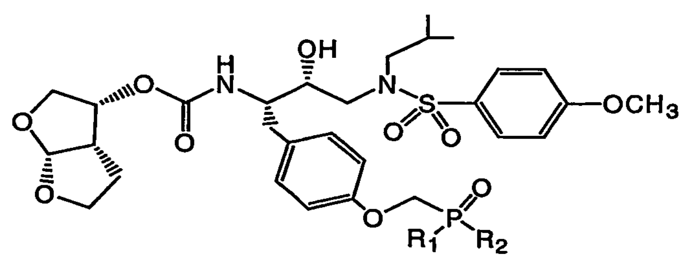
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
OE	OE	+++	+++	+	+	

P1-P-Bisamidates



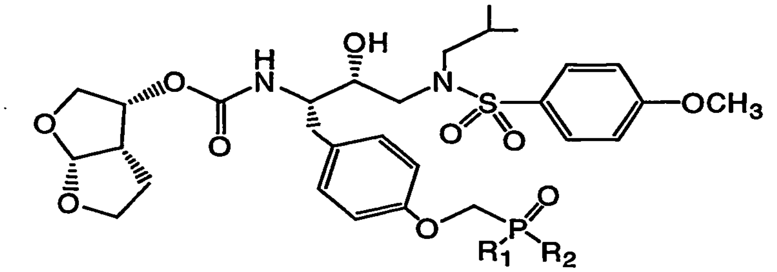
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
NHEt	NHEt	+++	++	–	–	
Gly-Et	Gly-Et	++	++			
Gly-Bu	Gly-Bu	+++	+++			
Ala-Et	Ala-Et	++	++		–	–
Ala-Bu	Ala-Bu	++	+++	+	–	
Aba-Et	Aba-Et	+++	+++			
Aba-Bu	Aba-Bu	+++	+++	++	+	
Val-Et	Val-Et	+	+++	–	–	
Leu-Et	Leu-Et	++	+++			
Leu-Bu	Leu-Bu	++	++	+	+	
Phe-Et	Phe-Et		+++			
Phe-Bu	Phe-Bu		+++			

P1-P-Bislactates



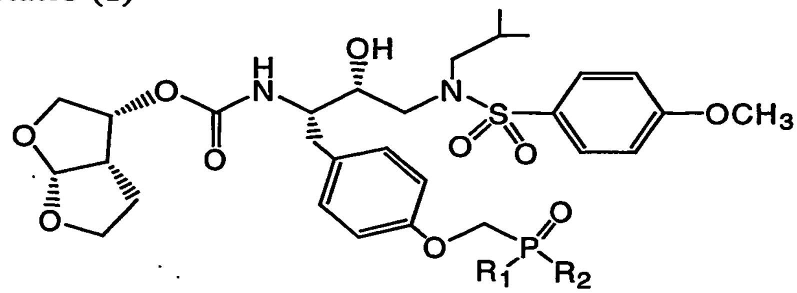
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
Glc-Et	Glc-Et	+++	+	–	–	
Lac-Et	Lac-Et	++	++	–	–	
Lac-iPr	Lac-iPr	++	+++		–	

P1-P-Monoamidates



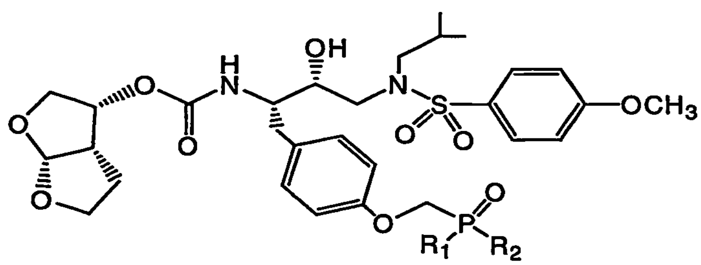
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
OPh	Gly-Bu	++	++	–	–	
OPh	Ala-Me	++	+++		–	
OPh	Ala-Et	+++	+++	–	–	
OPh	Ala-iPr	++	+++	–	–	
OPh	Ala-iPr	+++	+++			
OPh	Ala-iPr	++	+++			
OPh	(D)Ala-iPr	++	+++		–	
OPh	(D)Ala-iPr	+++	+++			
OPh	(D)Ala-iPr	+++	+++			
OPh	Ala-Bu	++	+++	–	–	
OPh	Ala-Bu	++	+++	–		
OPh	Ala-Bu	++	+++	–		
OPh	Aba-Et		+++			
OPh	Aba-Et		+++	–	–	
OPh	Aba-Et		++			
OPh	Aba-Bu		+++	+	–	
OPh	Aba-Bu		++	–	–	
OBn	Ala-Et	+++	+++	–	–	
OH	Ala-OH	+++	–			
OH	Ala-Bu		–			

P1-P-Monolactates (1)



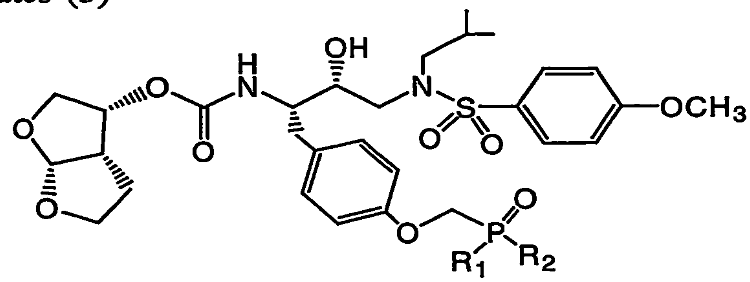
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I50V (#2) fold change	I84V/L90M fold change	CC_{50} μM
OPh	Glc-Et	+++	+++	–		–	
OPh	Lac-Me		++	–			
OPh	Lac-Et		+++	–	+	–	+
OPh	Lac-Et	+++	+++	–		–	
OPh	Lac-Et	++	+++	–		–	
OPh	Lac-iPr	++	+++	–		–	
OPh	Lac-iPr	+++	+++				
OPh	Lac-iPr	++	+++				
OPh	Lac-Bu	++	++			–	
OPh	Lac-Bu	++	++				
OPh	Lac-Bu	++	++				
OPh	Lac-EtMor		–				
OPh	Lac-PrMor		–				
OPh	(R)Lac-Me	+++	+++				
OPh	(R)Lac-Et	+++	+++	–		–	
OEt	Lac-Et		++				
OCH_2CF_3	Lac-Et		++				
OBn	Lac-Bn	++	++				
OBn	(R)Lac-Bn						
OH	Lac-OH	+++	+			–	
OH	(R)Lac-OH	++	+			–	

P1-P-Monolactates (2)



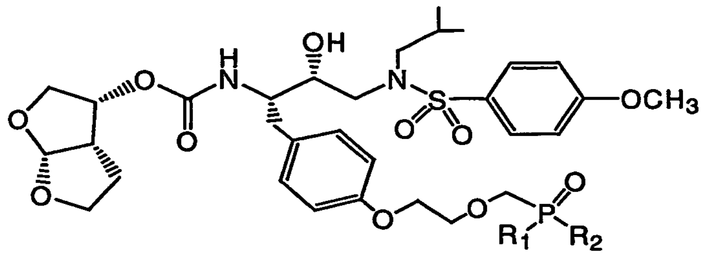
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
OPh	mix-Hba-Et	++	+++	+	–	
OPh	(S)Hba-Et	+	+++			
OPh	(S)Hba-tBu		+++			
OH	(S)Hba-OH	++				
OPh	(R)Hba-Et		+++			
OPh	(S)MeBut-Et		+++			
OPh	(R)MeBut-Et		+++			
OPh	DiMePro-Me	++				
OPh	(S)Lac-EtMor		–			
OPh	(S)Lac-PrMor		–			
OPh	(S)Lac-EtPip		++	–	–	

P1-P-Monolactates (3)



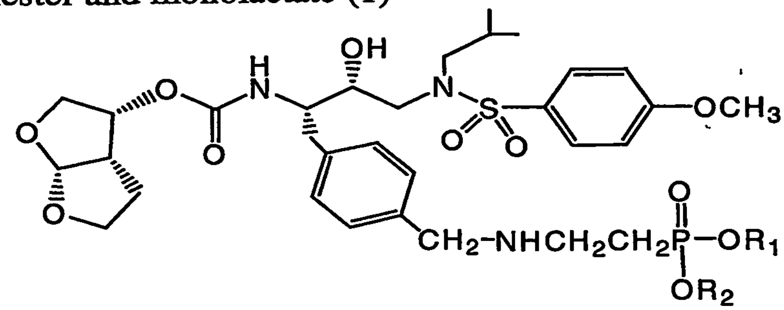
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
OPh—*o*-i-But	(S)Lac-Et		+++			
OPh—*p*-n-Oct	(S)Lac-Et		++			
OPh—*p*-n-But	(S)Lac-Et		+++			
OPh-*m*-COOBn	(S)Lac-Et		++			
OPh-*m*-COOH	(S)Lac-Et		++			
OPh-*m*-CH_2OH	(S)Lac-Et		++	–	–	
OPh-*m*-CH_2NH_2	(S)Lac-Et	++	++			
OPh-*m*-CH_2NMe_2	(S)Lac-Et		+			
OPh-*m*-CH_2Mor	(S)Lac-Et		++	–	–	
OPh-*m*-CH_2Pip	(S)Lac-Et		++			
OPh-*m*-CH_2NMeC2OM	(S)Lac-Et		++			
OPh-*o*-OEt	(S)Lac-Et		+++			
$ONMe_2$	(S)Lac-Et		++			
OPip	(S)Lac-Et		+			
OMor	(S)Lac-Et		–			

P1-C_2H_4-P-Monolactates



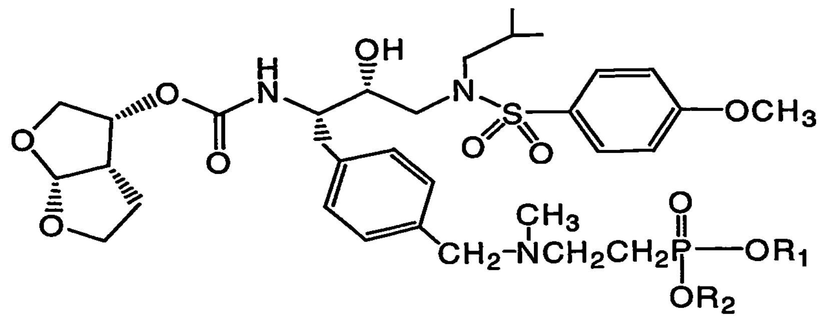
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
-OC_2H_4OBn			+++			
OEt	OEt		+++	–	–	
OPh	Lac-Et		++	–	–	
OH	OH	++				
OH	Lac	++				

P1-CH_2N-P-diester and monolactate (1)



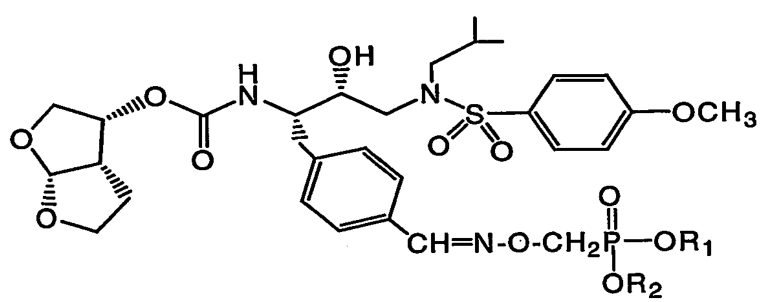
R_1	R_2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I50V (#2) fold change	I84V/L9M fold change	CC_{50} µM
Et	Et	++	+++		–		
H	H	++	-		+		
Ph	Lac-Et		++	–	++	–	
Ph	Lac-Et		+		+	–	–
Ph	Lac-Et		+		++	–	
Ph	Aba-Et		+		+	–	
Ph-oEt	Lac-Et	++	++	–	++	–	
Ph-dM	Lac-Et		+++		+	+	
Ph-dM	Lac-Pr		+++				
H	Lac	++					
Ph	Hba-Et		++		++	–	
Ph	Hba-Et		++		++	–	+
Ph	Hba-Et		++		++	–	
H	Hba	+					

P1-CH_2N-P-diester and monolactate (2)



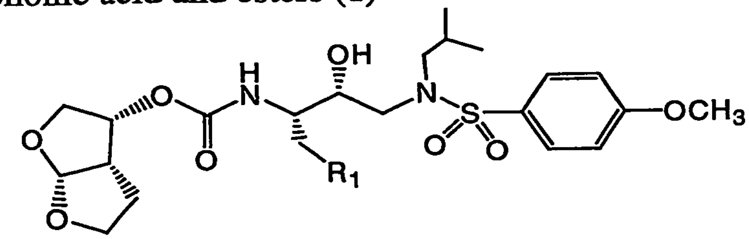
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50μM
Ph	Lac-Et	+	++	+	+	
H	H	++				

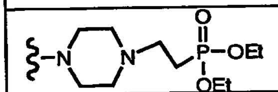
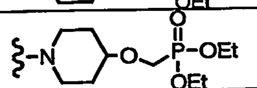
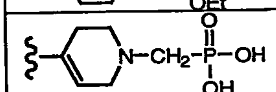
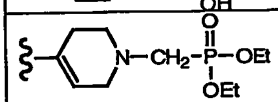
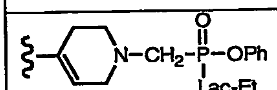
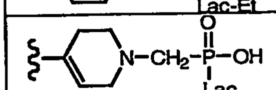
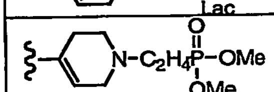
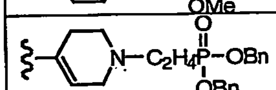
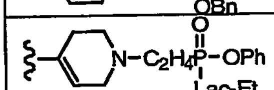
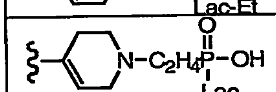
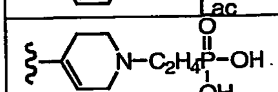
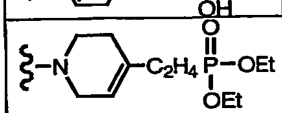
P1-CH_2N-P-diester and monolactate (3)



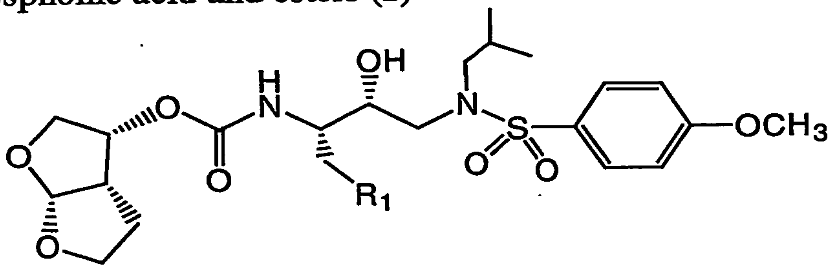
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50μM
Et	Et	++	+++		–	

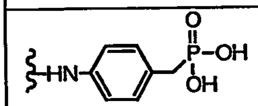
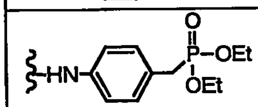
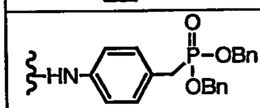
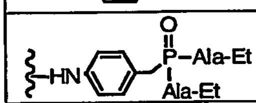
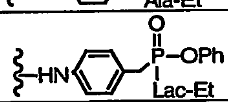
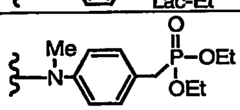
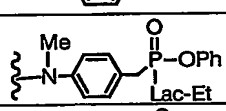
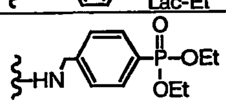
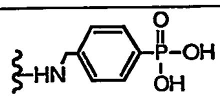
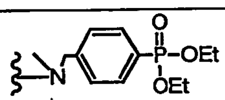
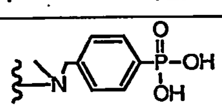
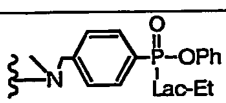
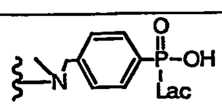
P1-N-P1-Phosphonic acid and esters (1)



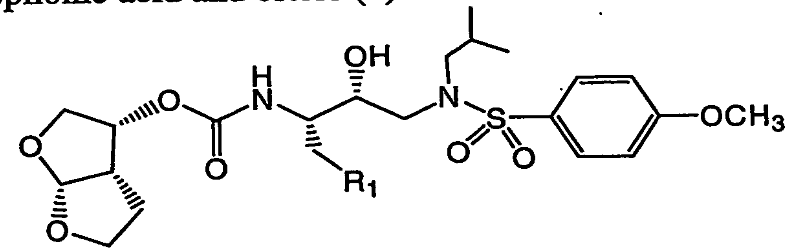
R1	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
	–	++			
	–	++			
	–				
	++	+++		+	
		–			
	–				
	+	++			
	++	+++		+	
		–			
		–			
	–				
	+	+++		+	

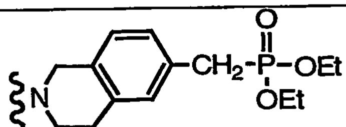
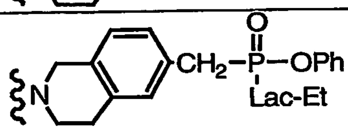
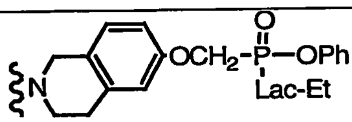
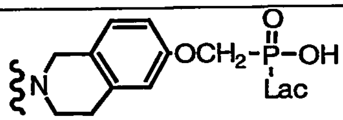
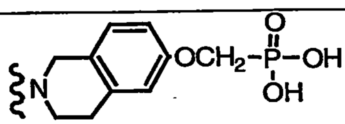
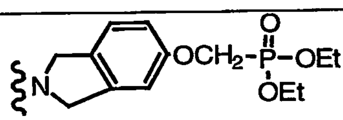
P1-N-P1-Phosphonic acid and esters (2)



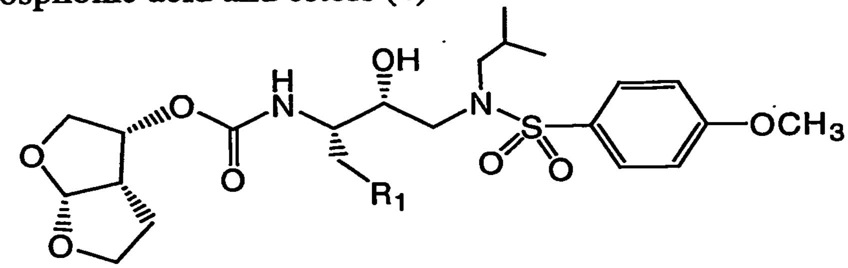
R1	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
	+	+		+	
	++	+++		+	
	++	+++			
	++	++		–	
		+++			
	++	+++		+	
		+++		–	
	–	+++		++	
	–				
	+	+++	+++	–	
	–				
		+++	++	+	
	–				

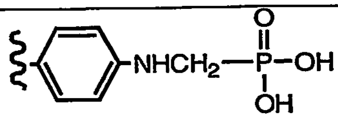
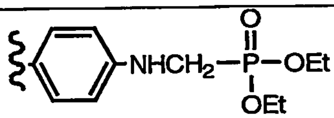
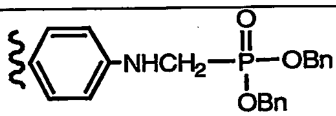
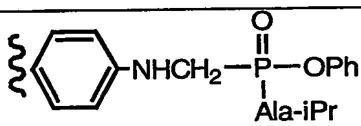
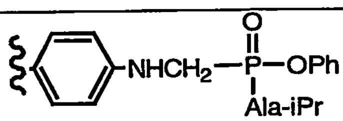
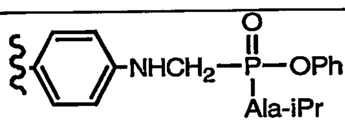
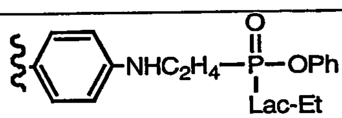
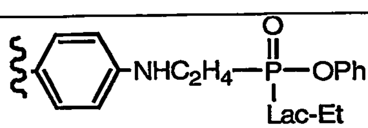
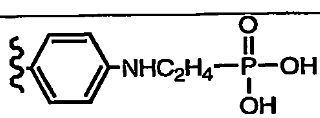
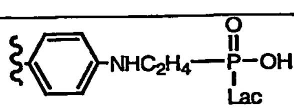
P1-N-P1-Phosphonic acid and esters (3)



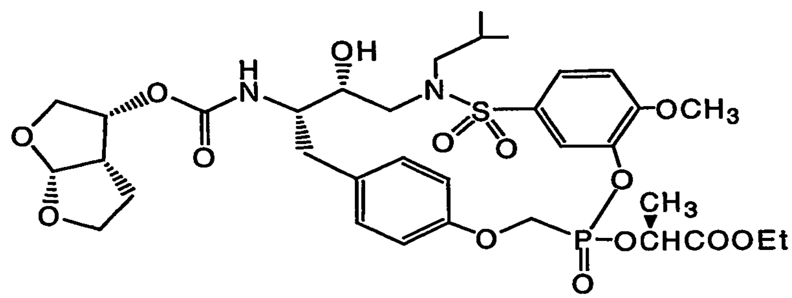
R1	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
	++	+++	+	+	
	+	++	+	+	
	+	++	+	+	
	+				
					
	–	–			

P1-N-P1-Phosphonic acid and esters (4)



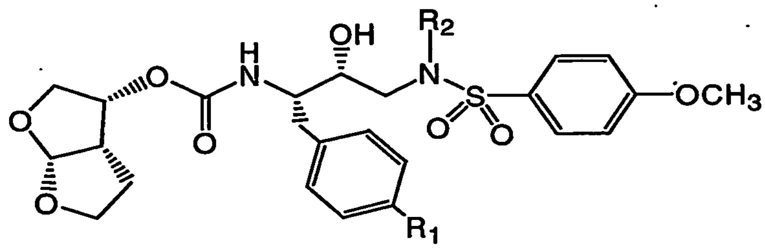
R1	Ki (pM)	EC$_{50}$ (nM)	I50V (#1) fold change	I84V/L90M fold change	CC$_{50}$ µM
	+++				
	+++	+++	–	–	
	++	+++	+	–	
	++	+++			
	++	++			
	+++	+++			
		+++	++	–	
		+++	++	–	
	++				
	++				

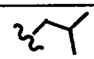
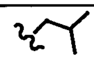
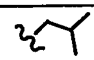
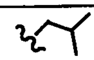
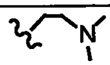
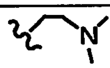
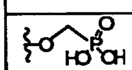
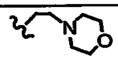
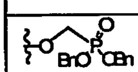
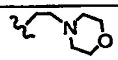
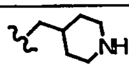
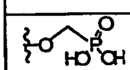
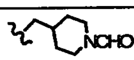
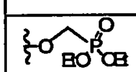
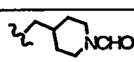
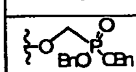
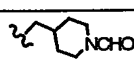
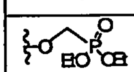
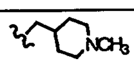
P1- P-cyclic monolactate



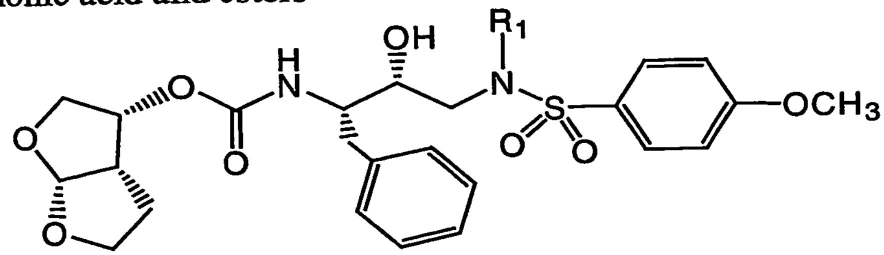
R_1	R_2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50}µM
		nd	nd			
		nd	nd			

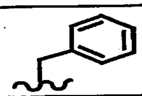
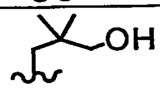
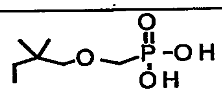
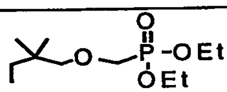
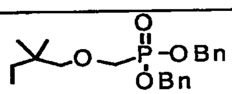
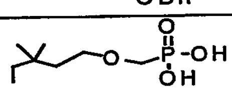
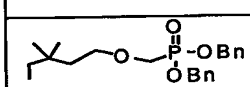
P1'-N-P1-Phosphonic acid and esters



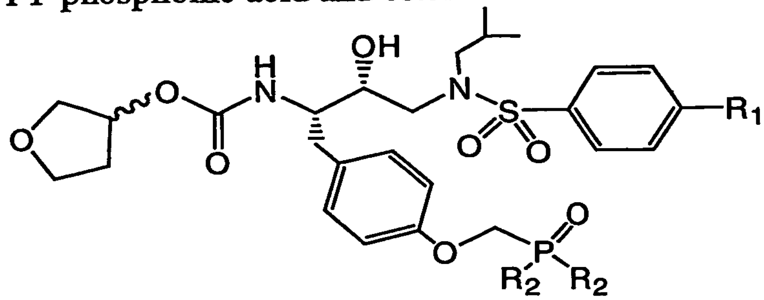
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
CH_3		++	+++	++	+	
OH			+++	–	–	
CH_2OH		+++	+++	–	–	
OBn		+++	+++	–	–	
OH		–	++	–	–	
OBn		–	+++		–	
		–	–	+	+	
		+	++	+	+	
OH		–	–			
		++	–			
		++	–			
		++	++			
		+	–			

P1'-Phosphonic acid and esters



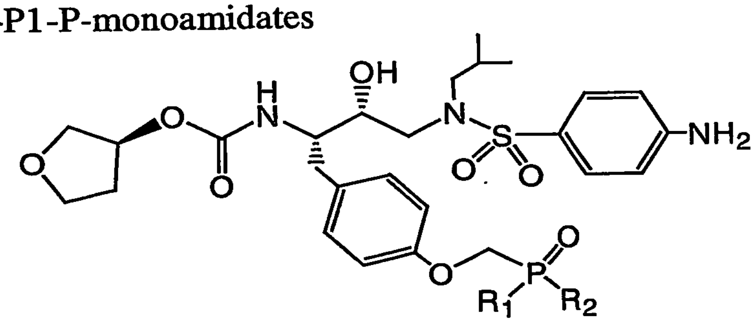
R1	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
	++	+++	+++	+++	
	+++	+++	+++	+++	
	++	+		+++	
	+++	+++		+++	
	+++	+++		++	
	++	++	++	++	
	++	+++	+++	+++	

P2-Monofuran-P1-phosphonic acid and esters



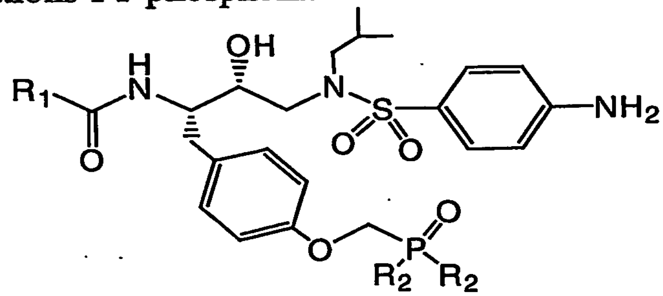
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
OMe	OH		–	+++	+++	
OMe	OEt	+++	+++	+++	++	
OMe	OBn		+++	++	++	
OMe	phenol	+++	+++	+++	+	
OMe	OEt	++	+++	+++	++	
NH_2	phenol	+	++	+	–	
NH_2	OH		–		+	
NH_2	OBn	++	++		+	

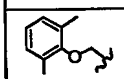
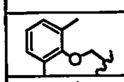
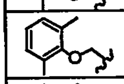
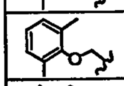
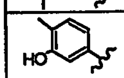
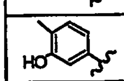
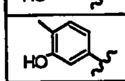
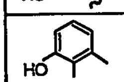
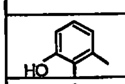
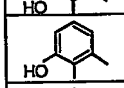
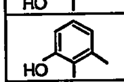
P2-Monofuran-P1-P-monoamidates



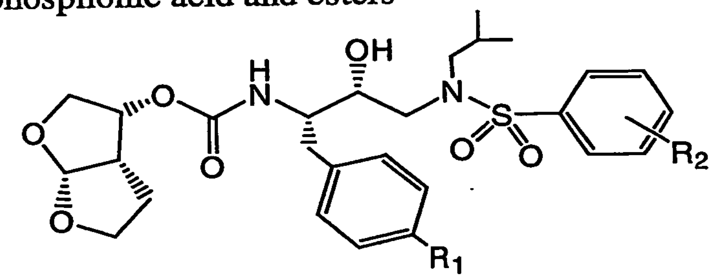
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
OPh	Ala-iPr	++	++		+	
OPh	Ala-iPr	++	++			
OPh	Ala-iPr	+	++			

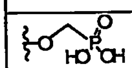
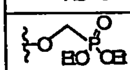
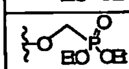
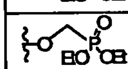
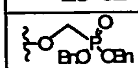
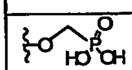
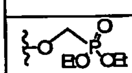
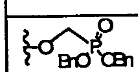
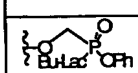
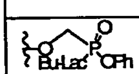
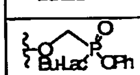
P2-Other modifications-P1-phosphonic acid and esters



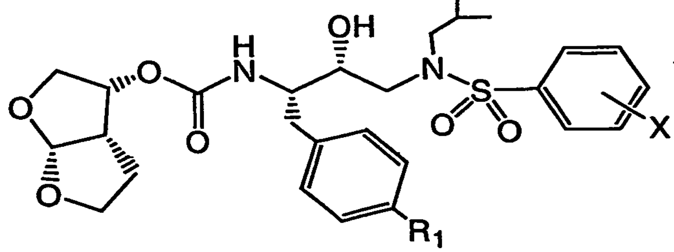
R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
	phenyl	+	+++	+++	++	
	phenol	+	++	++	+	
	OH	–	–	++	–	
	OBn	+	++	+	–	
	phenyl	+	++	+++	+	
	OH	+	–	++	+	
	OBn	+	++	+++	+	
	phenyl	–	++		++	
	phenol	+	+		–	
	OH	+	–	–	–	
	OBn	++	++	+	–	

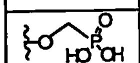
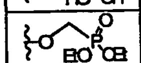
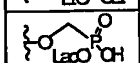
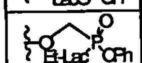
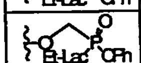
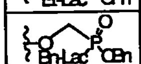
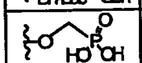
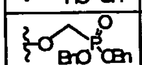
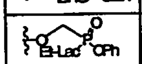
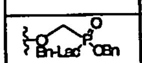
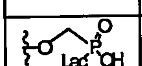
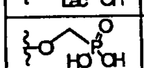
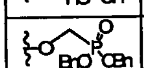
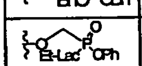
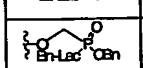
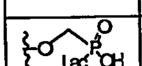
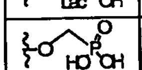
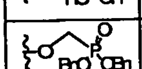
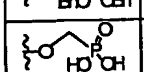
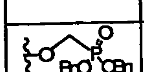
P2'-Amino-P1-phosphonic acid and esters

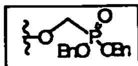
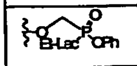
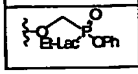


R1	R2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
OH	*p*-NH_2	++	++	–	–	
	*p*-NH_2	++	–	+	–	
	*p*-NH_2	++	+++		–	
	*p*-NO_2	++	+++		–	
	*p*-NHEt	++	+++		–	
	*p*-NH_2	++	+++	–	–	
OH	*m*-NH_2	++	++		–	
	*m*-NH_2	++	+		–	
	*m*-NH_2	++	++		–	
	*m*-NH_2	++	+++	–	–	
	*m*-NH_2	+	++	–	–	
	*m*-NH_2	++	++			
	*m*-NH_2	+	++			

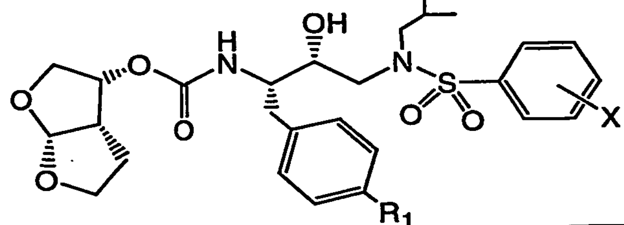
P2'-Substituted-P1-phosphonic acid and esters (1)

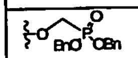
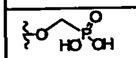
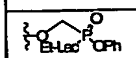
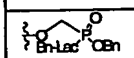
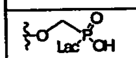
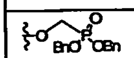
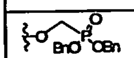
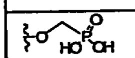
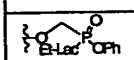
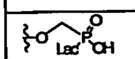
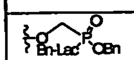
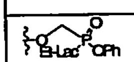
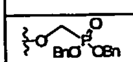
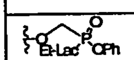
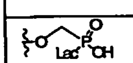
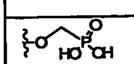
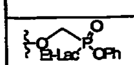
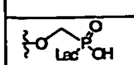
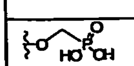


R1	X	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
	*p*-OH	+++	+			
	*p*-OH	+++	+++			
	*p*-OH	++				
	*p*-OH		+++		–	
	*p*-OBn		++			
	*p*-OBn		–			
	*p*-H	++	–			
	*p*-H	++	+++		+	
	*p*-H		+++	+	+	
	*p*-H		++			
	*p*-H	++				
	*p*-F	++	+			
	*p*-F	++	+++		+	
	*p*-F		+++	+	+	
	*p*-F		++	+	+	
	*p*-F	++				
	*p*-CF_3	+++	+			
	*p*-CF_3	++	+++		–	
	*p*-OCF_3	++	+			
	*p*-OCF_3	++	+++		+	

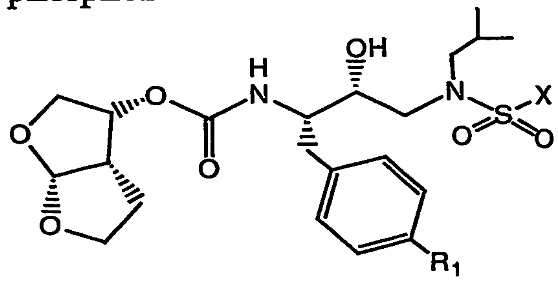
	*p*-CN	++	+++		–	
	*p*-Pip	–	–			
	*p*-Pip-Me	–	–			

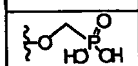
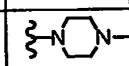
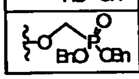
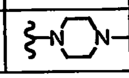
P2'-Substituted-P1-phosphonic acid and esters (2)



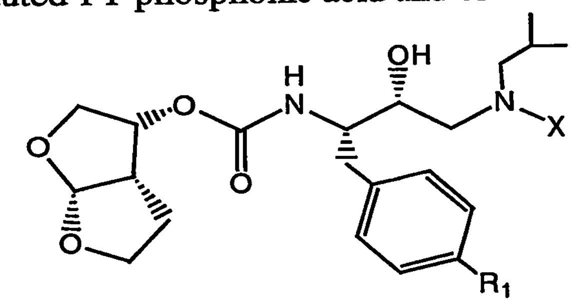
R1	X	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
	*m*-Py	++	+++			
	*m*-Py	++				
	*m*-Py	++	++	+	–	
	*m*-Py	++	++			
	*m*-Py	++				
	*m*-Py-Me+		+			
	*m*-Py-Me+		++			
	*m*-Py-oxide		++			
	*m*-Py-oxide	++				
	*m*-Py-oxide	++	++		–	
	*m*-Py-oxide	+				
	*m*-Py-oxide		–			
p-Py-oxide	*p*-OMe	++	–			
	*p*-CHO		+++			
	*p*-CHO		+++			
	*p*-CH2 OH		+++	–	–	
	*p*-CH2 OH	++				
	*p*-CH2 OH	++				
	*p*-CH2 Mor		++	–	–	
	*p*-CH2 Mor	–				
	*p*-CH2 Mor	–				

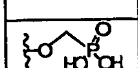
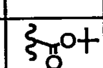
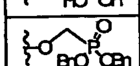
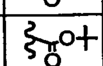
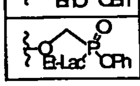
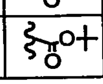
P2'-Alkylsulfonyl-P1-phosphonic acid and esters



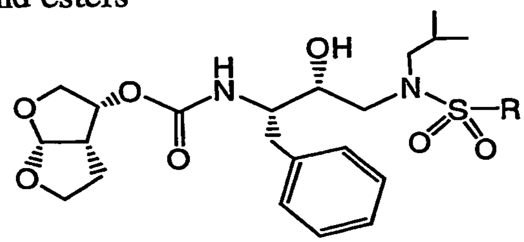
R1	X	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
		–	–			
		+	++			

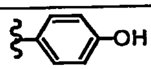
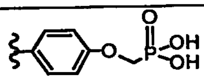
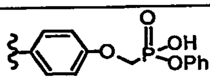
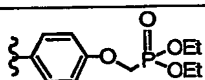
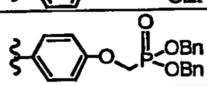
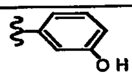
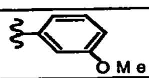
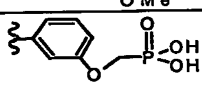
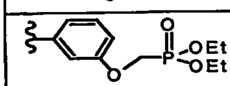
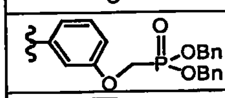
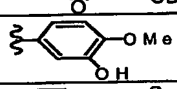
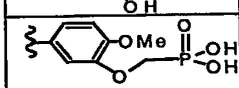
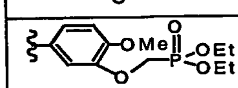
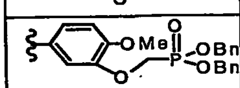
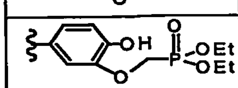
P2'-Carbonyl-substituted-P1-phosphonic acid and esters



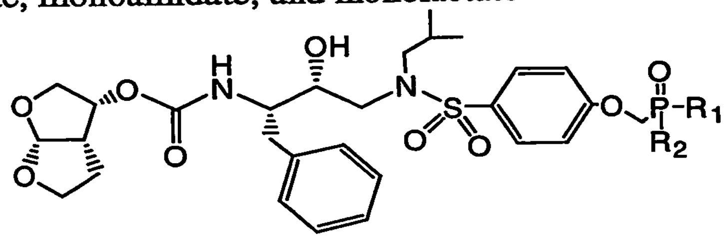
R1	X	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
		–				
		–	++			
			+			

P2'-Phosphonic acid and esters



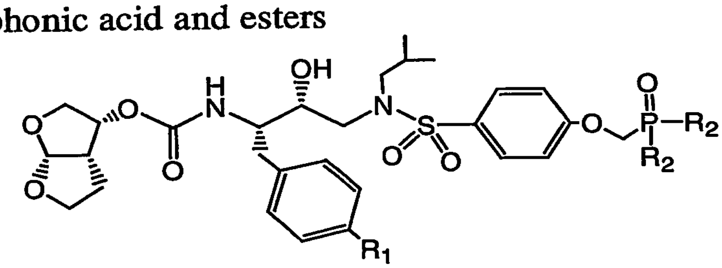
R	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
	+++	+++	–	–	
	+++	+	–	–	
	++	–			
	++	+++	++	++	
	+	++	+++	+++	
	+++	+++	+	+	
	+++	+++	+++	++	
	++	++	++	+	
	+++	+++	+++	++	
	++	+++	++	++	
	+++	+++	–	–	
	+++	++	+	–	
	+	++	+	+	
	–	+	+++	++	
	+	++	+	–	

P2'-P-Bisamidate, monoamidate, and monolactate



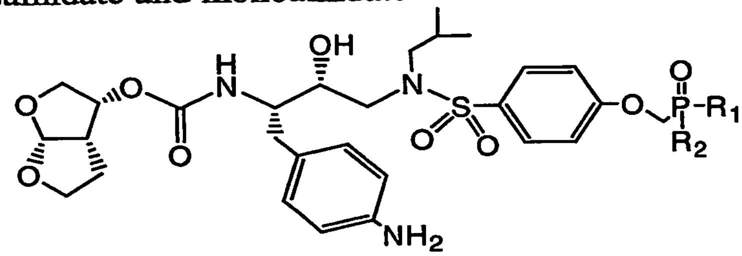
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
Ala-Bu	Ala-Bu	+	++	+	+	
OPh	Ala-iPr	++	++			
OPh	Lac-iPr	+	+			
OH	Ala-OH	++				

P1-N-P2'-Phosphonic acid and esters



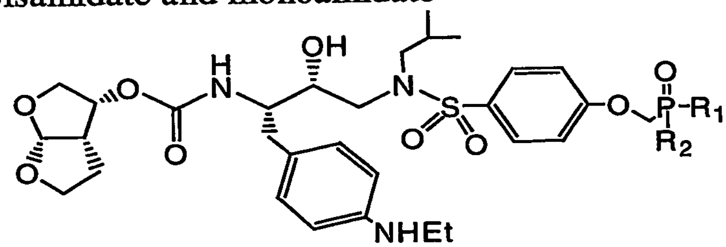
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
NO_2	phenol		+++	–		
NH_2	OH	++	-			
NH_2	OEt	+	++		++	
NH_2	OBn	+	+		+	
NMe_2	OEt	++	+++		++	
OH	OH	++	-			
OH	OBn	++	++			
$OC_2H_4NMe_2$	OH	+++	+			
OC_2H_4-NMe_2	OBn	++	++			

P1-N-P2'-P-Bisamidate and monoamidate



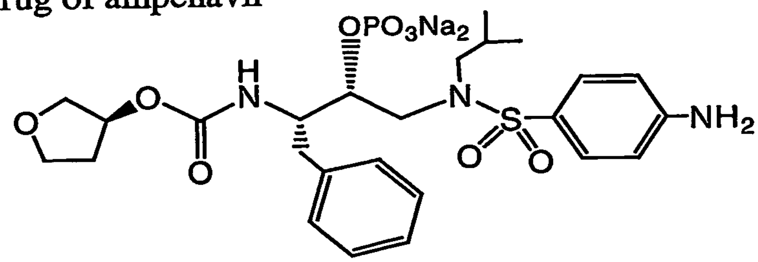
R_1	R_2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
Ala-Bu	Ala-Bu	+	+			
OPh	Ala-iPr	+	–			
OPh	Ala-iPr	++	–			

P1-NEt-P2'-P-Bisamidate and monoamidate



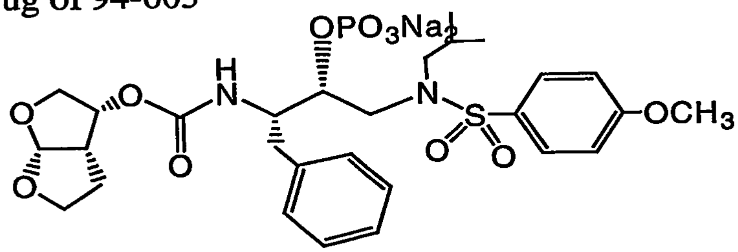
R_1	R_2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} μM
OPh	Ala-iPr	+	+			
OPh	Ala-iPr	+	+	–	–	

Phosphate prodrug of ampenavir



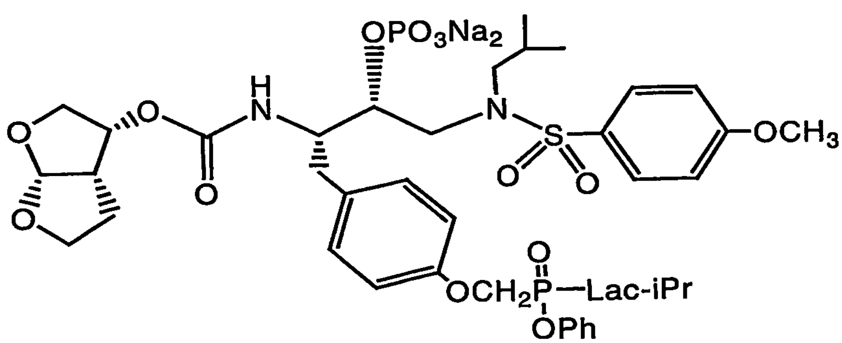
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
			++			

Phosphate prodrug of 94-003



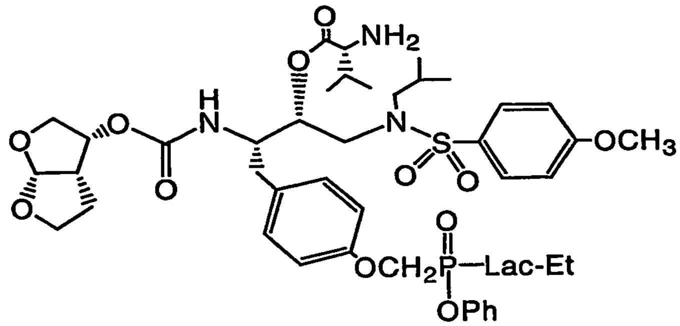
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
			+++			

Phosphate prodrug of GS77366 (P1-mono(S)Lac-iPr)



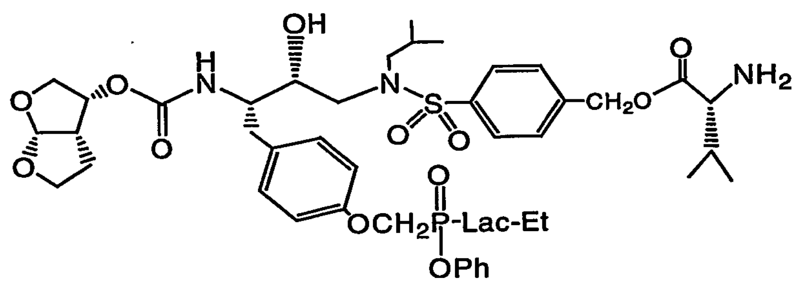
R1	R2	Ki (pM)	EC50 (nM)	I50V (#1) fold change	I84V/L90M fold change	CC50 µM
			+++			

Valine prodrug of (P1-mono(S)Lac-Et)



R_1	R_2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
			++			

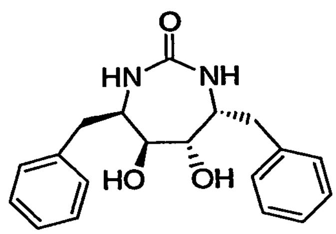
Valine prodrug of GS278053 (P1-mono(S)Lac-Et,P2'-CH_2OH)



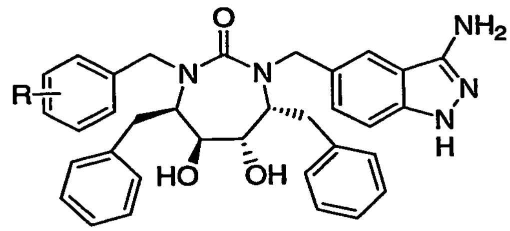
R_1	R_2	Ki (pM)	EC_{50} (nM)	I50V (#1) fold change	I84V/L90M fold change	CC_{50} µM
			++			

Table 11: Enzymatic and Cellular Activity Data

<u>Formula VIIIa CCLPPI activity</u>

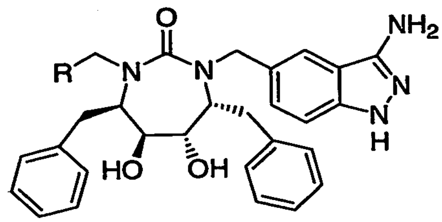


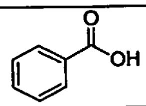
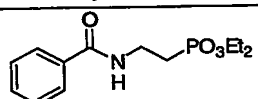
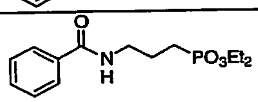
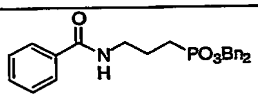
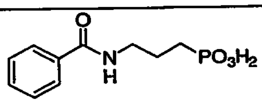
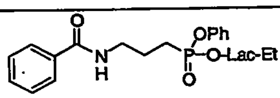
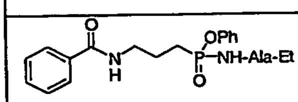
DMP-850

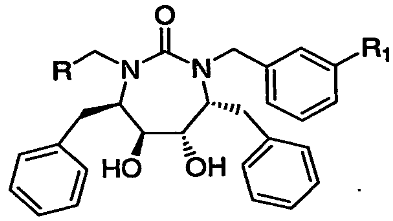


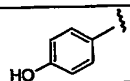
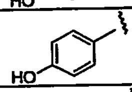
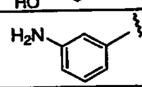
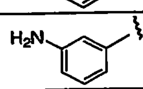
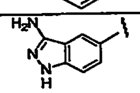
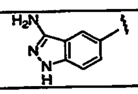
	Enzymatic assay			Cell-based assay (MT-4) EC_{50} / nM						
Structure, R	K_i (nM)	WT IC_{50}/ nM	84V90M IC_{50} / nM	WT	84V90M	30N 82I88D	48V54 V82A	48V54 V82S	48V82 A90M	46I50V
H (DMP-850)	0.033	3.0	9.1	165	819	82	82	73	45	88
p-OH	0.029	3.0	12	149	143	79	32	39	19	55
p-OBn	>5	353	781	2123	5312	1548	ND	ND	ND	ND
p-$OCH_2PO_3Bn_2$	>5	276	2042	2697	4963	2119	ND	ND	ND	ND
p-$OCH_2PO_3Et_2$	>5	627	1474	2480	>6000	1340	ND	ND	ND	ND
p-$OCH_2PO_3H_2$	>5	551	1657	>12000	ND	ND	ND	ND	ND	ND
m-OH	0.128	1.6	12	151	475	249	84			104
m-OBn	0.253	6.9	27	218	2422	82	709	ND	ND	601
m-$OCH_2PO_3Bn_2$ (N-iPr indazole)	1.54[a]	31	72	489	514	237	159	171	168	708
m-$OCH_2PO_3Bn_2$	0.177	18	43	898	>6000	705	2597	ND	ND	3121
m-$OCH_2PO_3Et_2$	1.93[a]	70	169	665	3005	93	513	ND	ND	857
m-$OCH_2PO_3H_2$	0.254	8.3	33	>12000	ND	ND	ND	ND	ND	ND

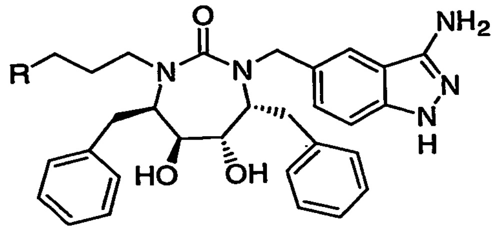
m-$OCH_2PO_3Ph_2$	0.543 a	10	42	1349	>600 0	1541	2183	ND	ND	3380
m-OCH_2PO_3HPh	0.644	17	65	1745	>600 0	ND	ND	ND	ND	ND
m-mono-Ala-Bu	0.858 a	6.6	39	1042	>600 0	425	790	ND	ND	797
m-mono-Ala-Et¶		35	68	1436	>600 0	219	734	ND	ND	1350
m-mono-Lac-Bu		15	34	2663	>600 0	1089	ND	ND	ND	ND
m-mono-Lac-Et		23	80	2609	>600 0	516	5923	ND	ND	>6000
m-bis-Ala-Bu	1.279 a	18	103	1079	>600 0	2362	1854	ND	ND	1536
m-bis-Ala-Et	1.987 a	31	202	5620	>600 0	1852	ND	ND	ND	ND



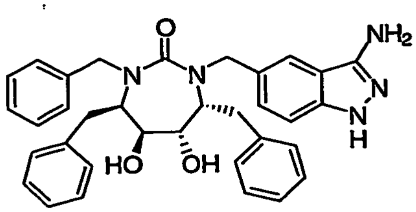
Structure, R	Enzymatic assay			Cell-based assay (MT-4) EC_{50} / nM						
	K_i (nM)	WT IC_{50}/ nM	84V90M IC_{50} / nM	WT	84V90M	30N 82I88D	48V54V82A	48V54V82S	48V82A90M	46I50V
H (DMP-850)	0.033	3.0	9.1	165	819	82	82	73	45	88
	0.091	3.4	27	1548	>6000	>6000	ND	ND	ND	ND
	0.354	3.3	25	168	909	750	277			489
	0.157	1.6	10	188	476	666	240			319
	0.044	5.0	27	491	387	234	238			192
	0.362	7.3	70	5141	>6000	4480	ND	ND	ND	ND
	0.112	1.4	6.4	603	1276	678	208			209
	<0.03	1.3	7.5	625	708	899	301			398



Structure, R1	Structure, R	Enzymatic assay K_i (nM)	Enzymatic assay WT IC_{50}/ nM	Enzymatic assay 84V90M IC_{50}/ nM	Cell-based assay (MT-4) EC_{50}/ nM WT	84V90M	30N82I88D	48V54V82A	48V54V82S	48V82A90M	46I50V
CO_2H			15	174	3055	>6000	887	ND	ND	ND	ND
$CONH(CH_2)_3PO_3Et_2$		0.009	1.1	12	65	311	74	80	75	74	85
CO_2H			18	299	2344	>6000	3360	ND	ND	ND	ND
$CONH(CH_2)_3PO_3Et_2$		<0.004	2.3	29	176	824	171	233	ND	ND	195
CO_2H		0.091	3.4	27	1548	>6000	>6000	ND	ND	ND	ND
$CONH(CH_2)_3PO_3Et_2$		0.157	1.6	10	188	476	666	240			319



	Enzymatic assay			Cell-based assay (MT-4) EC_{50}/ nM						
Structure, R	K_i (nM)	WT IC_{50}/ nM	84V90M IC_{50} / nM	WT	84V90M	30N 82I88D	48V54V82A	48V54V82S	48V82A90M	46I50V
CH_3 (DMP-851)	0.033	3.8	9.4	54	918	69	33	30	22	17
OH	0.65[a]	6.1	77	356	2791	669	294	ND	ND	683
$OCH_2PO_3Et_2$	1.230[a]	23	157	356	>6000	145	175	ND	ND	138
$OCH_2PO_3H_2$	0.809	59	137	1074	>6000	ND	ND	ND	ND	ND
O-mono-Lac-Et	>2.0	93	553	>6000	>6000	ND	ND	ND	ND	ND
O-mono-Lac-Bu	>2.0	25	249	>6000	>6000	ND	ND	ND	ND	ND
CH_2OH	0.017	2.8	31	253	1106	486	413	ND	ND	524
$CH_2OCH_2PO_3Et_2$	2.8	13	123	119	3295	267	430	ND	ND	789
$CH_2OCH_2PO_3H_2$		42	205	1757	>4243	ND	ND	ND	ND	ND



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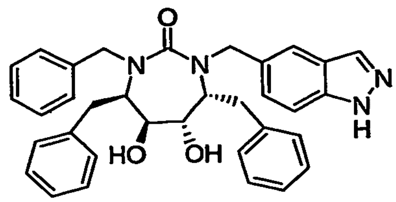

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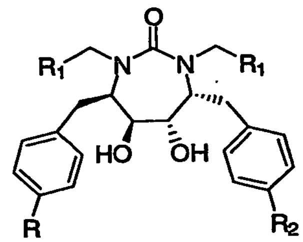
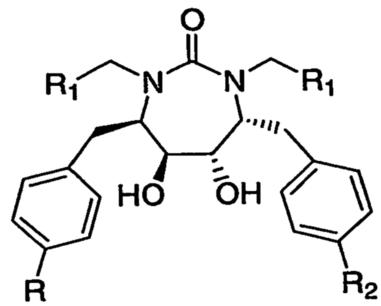
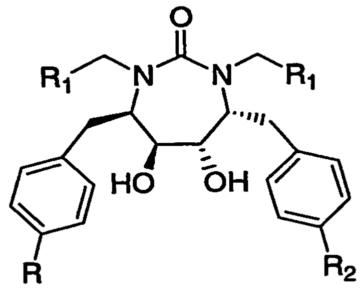


R	R1	R2	Enzymatic assay K_i (nM)	Enzymatic assay WT IC_{50}/ nM	Enzymatic assay 84V90M IC_{50} / nM	Cell-based assay (MT-4) EC_{50}/ nM WT	84V90M	30N82I88D	48V54V82A	48V54V82S	48V82A90M	46I50V
—	—	—	0.033	3.0	9.1	165	819	82	82	73	45	88
—	—	—	0.374	5.8	43.3	193	2312	281	705	ND	ND	772
H	Ph	H		34	631	2492	>600	3360	ND	ND	ND	ND
OH	Ph	OH		31	397	117	5609	756	2266	ND	ND	928
OH	Ph	OCH_2PO_3		9	40	33	791	92	807	1103	1429	53
H	Ph	OCH_2PO_3	0.656	3.9	48	107	2456	293	1438	1899	3292	589
H	Indazol	H	<0.01	2.5	13	11	22	<8	5.5	8	4	4.0
OH	Indazol	OH	0.012	0.6	3.5	>600	2728	7224	ND	ND	ND	ND
OH	Indazol	OCH_2PO_3	0.137	1.1	5.5	1698	1753	1998	ND	ND	ND	ND
H	Indazol	OCH_2PO_3	0.028	1.4	6.2	57	40	68	28	26	32	27

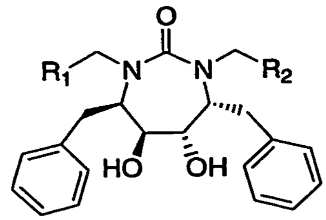


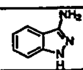
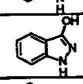
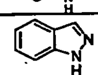
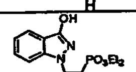
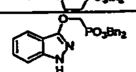
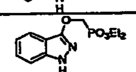
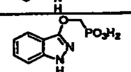
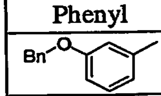
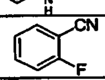
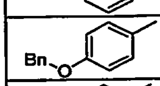
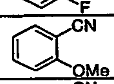
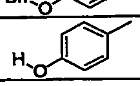
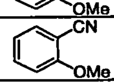
			Enzymatic assay		Cell-based assay (MT-4) EC_{50}/ nM							
R	R1	R2	K_i (nM)	WT IC_{50}/ nM	84V90M IC_{50}/ nM	WT	84V90M	30N82I88D	48V54V82A	48V54V82S	48V82A90M	46I50V
---	---	---	0.033	3.0	9.1	165	819	82	82	73	45	88
OH	Ph	$OCH_2PO_3Et_2$		9	40	33	791	92	807	1103	1429	53
H	Ph	$OCH_2PO_3Et_2$	0.656	3.9	48	107	2456	293	1438	1899	3292	589
OCH_3	Ph	$OCH_2PO_3Et_2$										
OH	Ph-pOH	$OCH_2PO_3Et_2$	<0.01	2.6	18	285	1912	211	986	ND	ND	1107
H	Ph-pOH	$OCH_2PO_3Et_2$	0.319	2.1	33	65	272	90	128	198	126	144
OCH_3	Ph-pOH	$OCH_2PO_3Et_2$	0.045	1.8	17	29	146	23	67	106	48	68
OH	Ph-mNH_2 /NHEt	$OCH_2PO_3Et_2$		8.7	67	286	1902	562	789	1781	684	239
H	Ph-mNH_2	$OCH_2PO_3Et_2$	0.126	3.4	39	65	328	16	168	146	74	46
OCH_3	Ph-mNH_2	$OCH_2PO_3Et_2$	<0.01	3.6	56	63	535	18	202	117	102	36
OCH_3	m-pyridine	$OCH_2PO_3Et_2$				115	765	106	1019	970	480	352



			Enzymatic assay			Cell-based assay (MT-4) EC_{50}/ nM						
R	R1	R2	K_i (nM)	WT IC_{50}/ nM	84V90M IC_{50}/ nM	WT	84V90M	30N82I88D	48V54V82A	48V54V82S	48V82A90M	46I50V
—	—	—	0.033	3.0	9.1	165	819	82	82	73	45	88
H	Ph-mNH$_2$	$OCH_2PO_3Et_2$	0.126	3.4	39	65	328	16	168	146	74	46
OCH_3	Ph-mNH$_2$	$OCH_2PO_3Et_2$	<0.01	3.6	56	63	535	18	202	117	102	36
OCH_3	Ph-mNH$_2$	$O(CH_2)_2PO_3Et_2$										
OCH_3	Ph-mNH$_2$	$OCONH(CH_2)_2PO_3Et_2$		11.3	116	74	2265	77	262	214	215	184
OCH_3	Ph-mNH$_2$	$OCONH(CH_2)PO_3Et_2$		9.9	85	58	2151	68	223	203	185	104
H	Ph-pOH	$OCH_2PO_3Et_2$	0.319	2.1	33	65	272	90	128	222	146	144
OCH_3	Ph-pOH	$OCH_2PO_3Et_2$	0.045	1.8	17	30	148	25	70	129	54	90
OCH_3	Ph-pOH	$OCONH(CH_2)_2PO_3Et_2$		6.6	49	33	495	31	74	51	55	223
—	—	—	0.033	3.0	9.1	165	819	82	82	73	45	88
H	Ph	$OCH_2PO_3Et_2$	0.656	3.9	48	107	2456	293	1438	1899	3292	589
H	Ph	OH	0.330	15	162	1261	>6000	2952	>6000			
H	Ph	$OCH_2PO_3Bn_2$	0.125	7.4	158	1769	>6000	3135	>6000			
H	Ph	$OCH_2PO_3H_2$	0.386	9.7	210	>600	>600	ND	ND			

						0	0					
H	Ph	Mono-lac-Et	0.120	6.6	56	1726	>600 0	2793	>6000			
H	Ph	Mono-Ala-Et		5	50	310	2943	238	2851	1948	2450	1250



R1	R2	Enzymatic assay K_i (nM)	Enzymatic assay WT IC_{50} / nM	Enzymatic assay 84V 90 M IC_{50} / nM	Cell-based assay (MT-4) EC_{50} / nM WT	84V 90M	30N 82I88 D	48V 54V 82A	48V54 V82S	48V82 A90M	46I5 0V
Phenyl		0.03	3.0	9.1	165	819	82	82	73	45	88
Phenyl		0.42	6.6	85	1226	>600	869	774	ND	ND	937
Phenyl		0.37	5.8	43.3	193	2312	281	705	ND	ND	772
Phenyl			109	>25	>6000	ND	ND	ND	ND	ND	ND
Phenyl											
Phenyl											
Phenyl											
		1.43	302	114	>6000	>600	ND	ND	ND	ND	ND
		>5	>25	ND	5949	ND	ND	ND	ND	ND	ND
		>5	130	348	2006	3121	ND	ND	ND	ND	ND

All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although certain embodiments have been described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.